P ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 08 June 2000 (08.06.00)	
International application No. PCT/US99/24407	**Applicant's or agent's file reference** 1133.011WO1
International filing date (day/month/year) 15 October 1999 (15.10.99)	**Priority date** (day/month/year) 16 October 1998 (16.10.98)
Applicant YANOFSKY, Martin, F.	

1. The designated Office is hereby notified of its election made:

[X] *in the demand filed with the International Preliminary Examining Authority on:*

15 May 2000 (15.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Col mbettes** **1211 Gen va 20, Switzerland**	Authorized officer C. Villet
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

US9924407

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

BASTIAN, Kevin, L.
Townsend and Townsend and Crew LLP
Two Embarcadero Center
8th Floor
San Francisco, CA 94111-3834
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 20 December 2000 (20.12.00)	
Applicant's or agent's file reference 1133.011WO1	**IMPORTANT NOTIFICATION**
International application No. PCT/US99/24407	**International filing date** (day/month/year) 15 October 1999 (15.10.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
VIKSNINS, Ann, S. Schwegman, Lundberg, Woessner & Kluth P.O. Box 2938 Minneapolis, MN 55402 United States of America		
	Telephone No. (612) 373-6900	
	Facsimile No. (612) 339-3061	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
BASTIAN, Kevin, L. Townsend and Townsend and Crew LLP Two Embarcadero Center 8th Floor San Francisco, CA 94111-3834 United States of America		
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Leitao
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 003732595

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 April 2000 (27.04.2000)

PCT

(10) International Publication Number
WO 00/23578 A3

(51) International Patent Classification[7]: C12N 15/82, 15/29, A01H 5/02

(21) International Application Number: PCT/US99/24407

(22) International Filing Date: 15 October 1999 (15.10.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/104,604 16 October 1998 (16.10.1998) US

(71) Applicant *(for all designated States except US)*: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 12th Floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **YANOFSKY, Martin, F.** [US/US]; 5039 Manor Ridge Lane, San Diego, CA 92130 (US).

(74) Agent: **VIKSNINS, Ann, S.**; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
7 December 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/23578 A3

(54) Title: METHODS OF SUPPRESSING FLOWERING IN TRANSGENIC PLANTS

(57) Abstract: The present invention provides a transgenic plant characterized by suppressed flowering. The transgenic plant contains a nucleic acid molecule including a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, wherein the nucleic acid molecule is heritable by progeny thereof.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 99/24407

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/29 A01H5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 13503 A (F B INVESTMENTS PTY LTD ;TEASDALE ROBERT DIXON (AU)) 2 April 1998 (1998-04-02)	1,2, 8-14, 23-25, 31,32
Y	abstract page 1, line 13 - line 19 page 3, line 10 - line 25 page 4, line 2 -page 5, line 11 page 6, line 24 -page 7, line 10 page 9, line 6 - line 8 --- -/--	1,2, 4-18, 23-29, 31,32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 September 2000	21. 9. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	MA ET AL.: "AGL1-AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes" GENES & DEVELOPMENT, vol. 5, no. 3, March 1991 (1991-03), pages 484-495, XP000905073 abstract; figure 3 ---	1,2,4,5, 8-16, 23-27, 31,32
Y	FEDERSPIEL ET AL: "Arabidopsis thaliana chromosome I BAC F316 genomic sequence, complete sequence" EMBL SEQUENCE DATABASE, 7 August 1997 (1997-08-07), XP002145696 HEIDELBERG DE Ac Ac002396 the whole document ---	1,2,6, 8-14,17, 23-25, 28,31,32
Y	WO 97 27287 A (UNIV CALIFORNIA) 31 July 1997 (1997-07-31) abstract; figure 10 ---	1,2, 7-14,18, 23-25, 29,31,32
A	MANDEL ET AL.: "Arabidopsis thaliana MADS-box (AGL9) mRNA, complet cds." EMBL SEQUENCE DATABASE, 29 August 1997 (1997-08-29), XP002145697 HEIDELBERG DE Ac AF015552 the whole document ---	6,17,28
A	ROUNSLEY ET AL.: "T33C10TF TAMU Arabidopsius thaliana genomic clone T33C10, genomic survey sequence" EMBL SEQUENCE DATABASE, 3 April 1998 (1998-04-03), XP002145698 HEIDELBERG DE Ac B97348 the whole document ---	28
A	US 5 554 798 A (LUNDQUIST RONALD C ET AL) 10 September 1996 (1996-09-10) cited in the application column 3, line 57 -column 4, line 23 --- -/--	1,13,23

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/US 99/24407

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PALMITER R D ET AL: "CELL LINEAGE ABLATION IN TRANSGENIC MICE BY CELL-SPECIFIC EXPRESSION OF A TOXIN GENE" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 50, 31 July 1987 (1987-07-31), pages 435-443, XP000198314 ISSN: 0092-8674 cited in the application abstract page 435, left-hand column, paragraph 2 -right-hand column, paragraph 1	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/24407

Box I Obs rvations wh re c rtain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1,2,4-18,23-29,31,32 (inventions 1-4)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark n Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 4, 15, 26

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL2 (Seq Id No 1).

2. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 5, 16, 27

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL4 (Seq Id No 2).

3. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 6, 17, 28

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL9 (Seq Id No 3).

4. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 7, 18, 29

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AP1 (Seq Id No 10).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is diphtheria toxic A chain.

6. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is RNase T1.

7. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is Barnase RNase.

8. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is ricin toxin A chain.

9. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is herpes simplex virus thymidine kinase.

10. Claims: partly: 13 and completly: 20-22

A method for producing a transgenic plant having suppressed flowering by intoducing a nucleic acid molecule comprising a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product by Agrobacterium-mediated transformation.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. .onal Application No
PCT/US 99/24407

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813503 A	02-04-1998	AU 4192997 A	17-04-1998
WO 9727287 A	31-07-1997	NONE	
US 5554798 A	10-09-1996	US 5484956 A	16-01-1996
		CA 2074355 A	23-07-1991
		CN 1054170 A,B	04-09-1991
		HU 62931 A	28-06-1993
		WO 9110725 A	25-07-1991
		US 5508468 A	16-04-1996
		US 6025545 A	15-02-2000
		US 5780708 A	14-07-1998
		US 5990390 A	23-11-1999
		RU 2114911 C	10-07-1998
		US 5538880 A	23-07-1996
		US 6013863 A	11-01-2000
		US 5538877 A	23-07-1996
		ZA 9100342 A	30-09-1992

PATENT COOPERATION TREATY

REC'D 13 FEB 2001
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1133.011WO1		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/24407	15/10/1999	16/10/1998

International Patent Classification (IPC) or national classification and IPC
C12N15/00

Applicant
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

CORRECTED VERSION

Date of submission of the demand	Date of completion of this report
15/05/2000	09.02.2001

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Heimann-Pohl, B

Telephone No. +49 89 2399 8713

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/24407

I. Basis f the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-30 as originally filed

Claims, No.:

1-33 as originally filed

Drawings, sheets:

1/43-43/43 as originally filed

Sequence listing part of the description, pages:

1-21, filed with the letter of 22.02.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 3, 19-22,30,33.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 3,19-22,30,33.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1,2,4-18, 23-29, 31,32.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,4-7,14-18,25-29,31,32
	No:	Claims	1,8-13, 23, 24
Inventive step (IS)	Yes:	Claims	
	No:	Claims	2,4-7,14-18,25-29,31,32
Industrial applicability (IA)	Yes:	Claims	1,2,4-18, 23-29,31,32
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1). The present application relates to transgenic plants, specifically trees important in wood production, containing a nucleic acid molecule including a floral organ selective regulatory element linked to a nucleotide sequence encoding a cytotoxic gene product.

2). Unity (Box IV)

The IPEA agrees with the ISA in regard to reasons for the non-unity objection which are the following:

Methods for modifying plants to increase vegetative growth of commercially valuable plant structures at the expense of non-essential and non-commercial structures are already known in the art. In WO9813503 (document D1) a method for producing a transgenic plant with enhanced vegetative growth is presented. In the method the plant is produced by introducing an expression cassette containing a structural gene for Barnase RNase under the control of a promoter that will cause specific expression of the gene in floral tissue. This leads to a decreased floral tissue growth and an increased vegetative tissue growth in the transgenic plant.

Due to the fact that nucleic acid constructs comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, and the use of said construct to produce a transgenic plant is already known from D1, the problem underlying the present application (as far as it had been subject to the Search Report) is the provision of further floral tissue specific promoters and transgenic plants containing them.

The requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the following groups of dependent claims:

1. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 4, 15, 26

An isolated nucleic acid molecule comprising a floral organ selective

regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AGL2 (SEQ ID NO: 1).

2. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 5, 16, 27

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AGL4 (SEQ ID NO: 2).

3. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 6, 17, 28

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AGL9 (SEQ ID NO: 3).

4. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 7, 18, 29

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AP1 (SEQ ID NO: 10).

In response to an invitation to pay additional fees or to restrict the claims, the

applicant has chosen to pay additional fees for inventions 2-4.

3). Prior Art

D1: WO 98 13503 A (F B INVESTMENTS PTY LTD ;TEASDALE ROBERT DIXON (AU)) 2 April 1998 (1998-04-02)
D2: MA ET AL.: 'AGL1-AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes' GENES & DEVELOPMENT, vol. 5, no. 3, March 1991 (1991-03), pages 484-495, XP000905073
D3: MANDEL ET AL.: 'Arabidopsis thaliana MADS-box (AGL9) mRNA, complet cds.' EMBL SEQUENCE DATABASE, 29 August 1997 (1997-08-29), XP002145697 HEIDELBERG DE
D4: WO 97 27287 A (UNIV CALIFORNIA) 31 July 1997 (1997-07-31)

D1 relates to a method of modifying a plant (specifically trees) to increase vegetative growth. The method involves a tissue specific promoter expressing during development of both male and female plant reproductive structures controlling a cytotoxic gene (Barnase). The tissue specific promoters used in D1, PrMADS1, 2 and 3, belong to the family of MADS-box genes showing homology to Arabidopsis ALG-2, ALG-4 and ALG-6. D1 further discloses a method for Agrobacterium-mediated transformation (pages 4-13, Example 3 and 6)

D2 reports that AGL-2 nd AGL4 are preferentially expressed in flowers. In situ RNA hybridization experiments with AGL-1 and AGL-2 showed that their mRNAs are detected in some floral organs but not in others.

D3 discloses that AGL-9 MADS-box gene is expressed in young flower primordia.

D4 discloses that AP1 and LFY contribute to establishing the floral meristem (paragraph bridging page 11- page 12).

4). Novelty (Box V)

The transgenic plant (claim 1, 11, 12, 23) the tissue derived from said plant of claim 1 (claim 8-10), the method for producing said plant (claim 23) and the

isolated nucleic acid molecule of claim 24 lack novelty, because due to the breadth of said claim subject matter disclosed in D1 falls under the scope of these claims (Art. 33 (2) PCT).

None of the documents discloses the subject matter of claims 2, 4-7, 14-18, 25-29, 31 and 32. The subject matter of these claims is therefore considered to be novel.

5). Inventive Step (Box V)

The problem underlying the present application, enhancing vegetative growth, can be derived from D1 which is regarded as the closest prior art document. The solution is the use of a tissue specific (floral organ specific) regulatory element which also is disclosed in D1. The solution of the present application is the use of alternative floral organ specific elements, AGL-2 (invention 1), AGL-4 (invention 2), AGL-9 (invention 3) and AP1 (invention 4). These alternative floral organ specific elements are all known in the art as being flower specific. The skilled person bearing in mind the teaching of D1 faced with the problem of providing alternative methods for enhancing vegetative growth in trees would have combined the teaching of D1 with either D2, D3 or D4 without the need of inventive skill and with a reasonable expectation of success. Consequently, the subject matter of claims 2, 4-7, 14-18, 25-29, 31 and 32 lacks an inventive step as required by Art. 33 (3) PCT.

6). Support, Art. 6 PCT (Box III)

The description of the present application relates to floral organ specific elements in connection with GUS expression (see Examples) thus the transgenic plants of claims 1, 2, 4-7 has not been reduced into practice.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference 1133.011WO1	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/24407	International filing date (day/month/year) 15/10/1999	Priority date (day/month/year) 16/10/1998

International Patent Classification (IPC) or national classification and IPC
C12N15/00

Applicant
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.

1. This International preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the International application
   - VIII ☐ Certain observations on the International application

Date of submission of the demand	Date of completion of this report
15/05/2000	09.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Heimann-Pohl, B Telephone No. +49 89 2399 8713

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/24407

I. Basis of th report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-30 as originally filed

Claims, No.:

1-33 as originally filed

Drawings, sheets:

1/43-43/43 as originally filed

Sequence listing part of the description, pages:

1-21, filed with the letter of 22.02.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 3, 19-22,30,33.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 3,19-22,30,33.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1,2,4-18, 23-29, 31,32.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,4-7,14-18,25-29,31,32
	No:	Claims	1,8-13, 23, 24
Inventive step (IS)	Yes:	Claims	
	No:	Claims	2,4-7,14-18,25-29,31,32
Industrial applicability (IA)	Yes:	Claims	1,2,4-18, 23-29,31,32
	No:	Claims	

2. Citations and explanations
see separate sheet

1). The pres nt application relates to transg nic plants, specifically trees important in wood production, containing a nucleic acid molecule including a floral organ selective regulatory element linked to a nucleotide sequence encoding a cytotoxic gene product.

2). Unity (Box IV)

The IPEA agrees with the ISA in regard to reasons for the non-unity objection which are the following:

Methods for modifying plants to increase vegetative growth of commercially valuable plant structures at the expense of non-essential and non-commercial structures are already known in the art. In WO9813503 (document D1) a method for producing a transgenic plant with enhanced vegetative growth is presented. In the method the plant is produced by introducing an expression cassette containing a structural gene for Barnase RNase under the control of a promoter that will cause specific expression of the gene in floral tissue. This leads to a decreased floral tissue growth and an increased vegetative tissue growth in the transgenic plant.

Due to the fact that nucleic acid constructs comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, and the use of said construct to produce a transgenic plant is already known from D1, the problem underlying the present application (as far as it had been subject to the Search Report) is the provision of further floral tissue specific promoters and transgenic plants containing them.

The requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the following groups of dependent claims:

1. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 4, 15, 26

An isolated nucleic acid molecule comprising a floral organ selective

regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AGL2 (SEQ ID NO: 1).

2. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 5, 16, 27

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AGL4 (SEQ ID NO: 2).

3. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 6, 17, 28

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AGL9 (SEQ ID NO: 3).

4. Claims : partly: 1, 2, 8-14, 23-25, 31, 32 and completely: 7, 18, 29

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operatively linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant containing said molecule and characterized by suppressed flowering and a method and kit for producing said plant, where the floral organ selective regulatory element is AP1 (SEQ ID NO: 10).

In response to an invitation to pay additional fees or to restrict the claims, the

applicant has chosen to pay additional fees for inventions 2-4.

3). Prior Art

D1: WO 98 13503 A (F B INVESTMENTS PTY LTD ;TEASDALE ROBERT DIXON (AU)) 2 April 1998 (1998-04-02)
D2: MA ET AL.: 'AGL1-AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes' GENES & DEVELOPMENT, vol. 5, no. 3, March 1991 (1991-03), pages 484-495, XP000905073
D3: MANDEL ET AL.: 'Arabidopsis thaliana MADS-box (AGL9) mRNA, complet cds.' EMBL SEQUENCE DATABASE, 29 August 1997 (1997-08-29), XP002145697 HEIDELBERG DE
D4: WO 97 27287 A (UNIV CALIFORNIA) 31 July 1997 (1997-07-31)

D1 relates to a method of modifying a plant (specifically trees) to increase vegetative growth. The method involves a tissue specific promoter expressing during development of both male and female plant reproductive structures controlling a cytotoxic gene (Barnase). The tissue specific promoters used in D1, PrMADS1, 2 and 3, belong to the family of MADS-box genes showing homology to Arabidopsis ALG-2, ALG-4 and ALG-6. D1 further discloses a method for Agrobacterium-mediated transformation (pages 4-13, Example 3 and 6)

D2 reports that AGL-2 nd AGL4 are preferentially expressed in flowers. In situ RNA hybridization experiments with AGL-1 and AGL-2 showed that their mRNAs are detected in some floral organs but not in others.

D3 discloses that AGL-9 MADS-box gene is expressed in young flower primordia.

D4 discloses that AP1 and LFY contribute to establishing the floral meristem (paragraph bridging page 11- page 12).

4). Novelty (Box V)

The transgenic plant (claim 1, 11, 12, 23) the tissue derived from said plant of claim 1 (claim 8-10), the method for producing said plant (claim 23) and the

isolated nucleic acid molecule of claim 24 lack novelty, because due to the breadth of said claim subject matter disclosed in D1 falls under the scope of these claims (Art. 33 (2) PCT).

None of the documents discloses the subject matter of claims 2, 4-7, 14-18, 25-29, 31 and 32. The subject matter of these claims is therefore considered to be novel.

5). Inventive Step (Box V)

The problem underlying the present application, enhancing vegetative growth, can be derived from D1 which is regarded as the closest prior art document. The solution is the use of a tissue specific (floral organ specific) regulatory element which also is disclosed in D1. The solution of the present application is the use of alternative floral organ specific elements, AGL-2 (invention 1), AGL-4 (invention 2), AGL-9 (invention 3) and AP1 (invention 4). These alternative floral organ specific elements are all known in the art as being flower specific. The skilled person bearing in mind the teaching of D1 faced with the problem of providing alternative methods for enhancing vegetative growth in trees would have combined the teaching of D1 with either D2, D3 or D4 without the need of inventive skill and with a reasonable expectation of success. Consequently, the subject matter of claims 2, 4-7, 14-18, 25-29, 31 and 32 lacks an inventive step as required by Art. 33 (3) PCT.

6). Support, Art. 6 PCT (Box III)

The description of the present application relates to floral organ specific elements in connection with GUS expression (see Examples) thus the transgenic plants of claims 1, 2, 4-7 has not been reduced into practice.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
1133.011W01		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/24407	15/10/1999	16/10/1998
Applicant		
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/24407

Box I Observations where certain claims were found unsearchable (Continuation of it m 1 f first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1,2,4-18,23-29,31,32 (inventions 1-4)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark n Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 4, 15, 26

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL2 (Seq Id No 1).

2. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 5, 16, 27

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL4 (Seq Id No 2).

3. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 6, 17, 28

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL9 (Seq Id No 3).

4. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 7, 18, 29

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AP1 (Seq Id No 10).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is diphtheria toxic A chain.

6. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is RNase T1.

7. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is Barnase RNase.

8. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is ricin toxin A chain.

9. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is herpes simplex virus thymidine kinase.

10. Claims: partly: 13 and completly: 20-22

A method for producing a transgenic plant having suppressed flowering by intoducing a nucleic acid molecule comprising a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product by Agrobacterium-mediated transformation.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/24407

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/29 A01H5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 13503 A (F B INVESTMENTS PTY LTD ;TEASDALE ROBERT DIXON (AU)) 2 April 1998 (1998-04-02)	1,2, 8-14, 23-25, 31,32
Y	abstract page 1, line 13 - line 19 page 3, line 10 - line 25 page 4, line 2 -page 5, line 11 page 6, line 24 -page 7, line 10 page 9, line 6 - line 8 --- -/--	1,2, 4-18, 23-29, 31,32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 September 2000	21. 9. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Ceder, O

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	MA ET AL.: "AGL1-AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes" GENES & DEVELOPMENT, vol. 5, no. 3, March 1991 (1991-03), pages 484-495, XP000905073 abstract; figure 3 ---	1,2,4,5, 8-16, 23-27, 31,32
Y	FEDERSPIEL ET AL: "Arabidopsis thaliana chromosome I BAC F316 genomic sequence, complete sequence" EMBL SEQUENCE DATABASE, 7 August 1997 (1997-08-07), XP002145696 HEIDELBERG DE Ac Ac002396 the whole document ---	1,2,6, 8-14,17, 23-25, 28,31,32
Y	WO 97 27287 A (UNIV CALIFORNIA) 31 July 1997 (1997-07-31) abstract; figure 10 ---	1,2, 7-14,18, 23-25, 29,31,32
A	MANDEL ET AL.: "Arabidopsis thaliana MADS-box (AGL9) mRNA, complet cds." EMBL SEQUENCE DATABASE, 29 August 1997 (1997-08-29), XP002145697 HEIDELBERG DE Ac AF015552 the whole document ---	6,17,28
A	ROUNSLEY ET AL.: "T33C10TF TAMU Arabidopsius thaliana genomic clone T33C10, genomic survey sequence" EMBL SEQUENCE DATABASE, 3 April 1998 (1998-04-03), XP002145698 HEIDELBERG DE Ac B97348 the whole document ---	28
A	US 5 554 798 A (LUNDQUIST RONALD C ET AL) 10 September 1996 (1996-09-10) cited in the application column 3, line 57 -column 4, line 23 --- -/--	1,13,23

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PALMITER R D ET AL: "CELL LINEAGE ABLATION IN TRANSGENIC MICE BY CELL-SPECIFIC EXPRESSION OF A TOXIN GENE" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 50, 31 July 1987 (1987-07-31), pages 435-443, XP000198314 ISSN: 0092-8674 cited in the application abstract page 435, left-hand column, paragraph 2 -right-hand column, paragraph 1	1

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/24407

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813503 A	02-04-1998	AU 4192997 A	17-04-1998
WO 9727287 A	31-07-1997	NONE	
US 5554798 A	10-09-1996	US 5484956 A	16-01-1996
		CA 2074355 A	23-07-1991
		CN 1054170 A,B	04-09-1991
		HU 62931 A	28-06-1993
		WO 9110725 A	25-07-1991
		US 5508468 A	16-04-1996
		US 6025545 A	15-02-2000
		US 5780708 A	14-07-1998
		US 5990390 A	23-11-1999
		RU 2114911 C	10-07-1998
		US 5538880 A	23-07-1996
		US 6013863 A	11-01-2000
		US 5538877 A	23-07-1996
		ZA 9100342 A	30-09-1992

. ATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
Schwegman, Lundberg, Woessner
& Kluth
Attn. VIKSNINS, Ann S
P.O.Box 2938
Minneapolis, Minnesota 55402
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)* 21/09/2000

Applicant's or agent's file reference
1133.011WO1

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/US 99/24407

International filing date *(day/month/year)* 15/10/1999

Applicant
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Schwegman, Lundberg, Woessner & Kluth, P.A.

Name and mailing address of the International Searching Authority
European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Peggy Frenzel

SEP 27 2000
RECEIVED

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continu d)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
1133.011WO1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/24407	15/10/1999	16/10/1998
Applicant		
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,** ✓

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/24407

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1,2,4-18,23-29,31,32 (inventions 1-4)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 4, 15, 26

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL2 (Seq Id No 1).

2. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 5, 16, 27

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL4 (Seq Id No 2).

3. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 6, 17, 28

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AGL9 (Seq Id No 3).

4. Claims: partly: 1, 2, 8-14, 23-25, 31, 32 and completly: 7, 18, 29

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the floral organ selective regulatory element is AP1 (Seq Id No 10).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is diphtheria toxic A chain.

6. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is RNase T1.

7. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is Barnase RNase.

8. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is ricin toxin A chain.

9. Claims: partly: 1, 3, 8-13, 19, 23, 24, 30, 31, 33

An isolated nucleic acid molecule comprising a floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product, a transgenic plant characterised by suppressed flowering containing it and a method and kit for producing the plant, where the cytotoxic gene product is herpes simplex virus thymidine kinase.

10. Claims: partly: 13 and completly: 20-22

A method for producing a transgenic plant having suppressed flowering by intoducing a nucleic acid molecule comprising a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

floral organ selective regulatory element operativly linked to a nucleotide sequence encoding a cytotoxic gene product by Agrobacterium-mediated transformation.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/24407

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/29 A01H5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 13503 A (F B INVESTMENTS PTY LTD ;TEASDALE ROBERT DIXON (AU)) 2 April 1998 (1998-04-02)	1,2, 8-14, 23-25, 31,32
Y	abstract page 1, line 13 - line 19 page 3, line 10 - line 25 page 4, line 2 -page 5, line 11 page 6, line 24 -page 7, line 10 page 9, line 6 - line 8 --- -/--	1,2, 4-18, 23-29, 31,32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 September 2000	21. 9. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Ceder, O

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	MA ET AL.: "AGL1-AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes" GENES & DEVELOPMENT, vol. 5, no. 3, March 1991 (1991-03), pages 484-495, XP000905073 abstract; figure 3 ---	1,2,4,5, 8-16, 23-27, 31,32
Y	FEDERSPIEL ET AL: "Arabidopsis thaliana chromosome I BAC F316 genomic sequence, complete sequence" EMBL SEQUENCE DATABASE, 7 August 1997 (1997-08-07), XP002145696 HEIDELBERG DE Ac Ac002396 the whole document ---	1,2,6, 8-14,17, 23-25, 28,31,32
Y	WO 97 27287 A (UNIV CALIFORNIA) 31 July 1997 (1997-07-31) abstract; figure 10 ---	1,2, 7-14,18, 23-25, 29,31,32
A	MANDEL ET AL.: "Arabidopsis thaliana MADS-box (AGL9) mRNA, complet cds." EMBL SEQUENCE DATABASE, 29 August 1997 (1997-08-29), XP002145697 HEIDELBERG DE Ac AF015552 the whole document ---	6,17,28
A	ROUNSLEY ET AL.: "T33C10TF TAMU Arabidopsius thaliana genomic clone T33C10, genomic survey sequence" EMBL SEQUENCE DATABASE, 3 April 1998 (1998-04-03), XP002145698 HEIDELBERG DE Ac B97348 the whole document ---	28
A	US 5 554 798 A (LUNDQUIST RONALD C ET AL) 10 September 1996 (1996-09-10) cited in the application column 3, line 57 -column 4, line 23 --- -/--	1,13,23

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PALMITER R D ET AL: "CELL LINEAGE ABLATION IN TRANSGENIC MICE BY CELL-SPECIFIC EXPRESSION OF A TOXIN GENE" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 50, 31 July 1987 (1987-07-31), pages 435-443, XP000198314 ISSN: 0092-8674 cited in the application abstract page 435, left-hand column, paragraph 2 -right-hand column, paragraph 1	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/24407

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813503 A	02-04-1998	AU 4192997 A	17-04-1998
WO 9727287 A	31-07-1997	NONE	
US 5554798 A	10-09-1996	US 5484956 A	16-01-1996
		CA 2074355 A	23-07-1991
		CN 1054170 A,B	04-09-1991
		HU 62931 A	28-06-1993
		WO 9110725 A	25-07-1991
		US 5508468 A	16-04-1996
		US 6025545 A	15-02-2000
		US 5780708 A	14-07-1998
		US 5990390 A	23-11-1999
		RU 2114911 C	10-07-1998
		US 5538880 A	23-07-1996
		US 6013863 A	11-01-2000
		US 5538877 A	23-07-1996
		ZA 9100342 A	30-09-1992

ID AC002396 standard; DNA; PLN; 122358 BP.
AC AC002396;
SV AC002396.1
DT 07-AUG-1997 (Rel. 52, Created)
DT 03-MAR-2000 (Rel. 62, Last updated, Version 5)
DE Arabidopsis thaliana chromosome I BAC F3I6 genomic sequence, complete
DE sequence.
KW HTG.
OS Arabidopsis thaliana (thale cress)
OC Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta;
OC Magnoliophyta; eudicotyledons; Rosidae; eurosids II; Brassicales;
OC Brassicaceae; Arabidopsis.
RN [1]
RP 1-122358
RA Federspiel N.A., Palm C.J., Conway A.B., Kurtz D.B., Conway A.R., Au M.,
RA Araujo R., Buehler E., Dewar K., Feng J., Kim C., Li Y., Oji O.,
RA Osborne B.I., Shinn P., Sun H., Toriumi M., Vysotskaia V.S., Yu G.,
RA Ecker J., Theologis A., Davis R.W.;
RT ;
RL Unpublished.
RN [2]
RP 1-122358
RA Federspiel N.A., Palm C.J., Conway A.B., Kurtz D.B., Conway A.R., Au M.,
RA Araujo R., Brendel V., Buehler E., Dewar K., Feng J., Kim C., Li Y.,
RA Oji O., Osborne B.I., Shinn P., Sun H., Toriumi M., Vyotskaia V., Yu G.,
RA Ecker J., Theologis A., Davis R.W.;
RT ;
RL Submitted (31-JUL-1997) to the EMBL/GenBank/DDBJ databases.
RL Biochemistry, Stanford University/DNA Sequencing and Technology Center, 855
RL California Avenue, Palo Alto, CA 94304, USA
RN [3]
RP 1-122358
RA Federspiel N.A., Palm C.J., Conway A.B., Kurtz D.B., Conway A.R., Au M.,
RA Araujo R., Buehler E., Dewar K., Feng J., Kim C., Li Y., Oji O.,
RA Osborne B.I., Shinn P., Sun H., Toriumi M., Vyotskaia V., Yu G., Ecker J.,
RA Theologis A., Davis R.W.;
RT ;
RL Submitted (06-JAN-1998) to the EMBL/GenBank/DDBJ databases.
RL Stanford DNA Sequencing and Technology Center, Stanford University, 855
RL California Avenue, Palo Alto, CA 94304, USA
RN [4]
RP 1-122358
RA Federspiel N.A., Palm C.J., Conway A.B., Kurtz D.B., Conway A.R., Au M.,
RA Araujo R., Buehler E., Dewar K., Feng J., Kim C., Li Y., Oji O.,
RA Osborne B.I., Shinn P., Sun H., Toriumi M., Vyotskaia V., Yu G., Ecker J.,
RA Theologis A., Davis R.W.;
RT ;
RL Submitted (04-FEB-1998) to the EMBL/GenBank/DDBJ databases.
RL DNA Sequencing and Technology Center, Stanford University, 855

California
RL Avenue, Palo Alto, CA 94304, USA . . .

SCORES Init1: 73170 Initn: 73170 Opt: 74436 z-scor : 90851.8 E():
0
99.8% identity in 14943 bp overlap

```
                                          14939     14929     14919
Sa256076_000                                 TCTTGGGAATATTTTTTTGGCAGAAACAAA

                                                  ||||||||||||||||||||||||||||||
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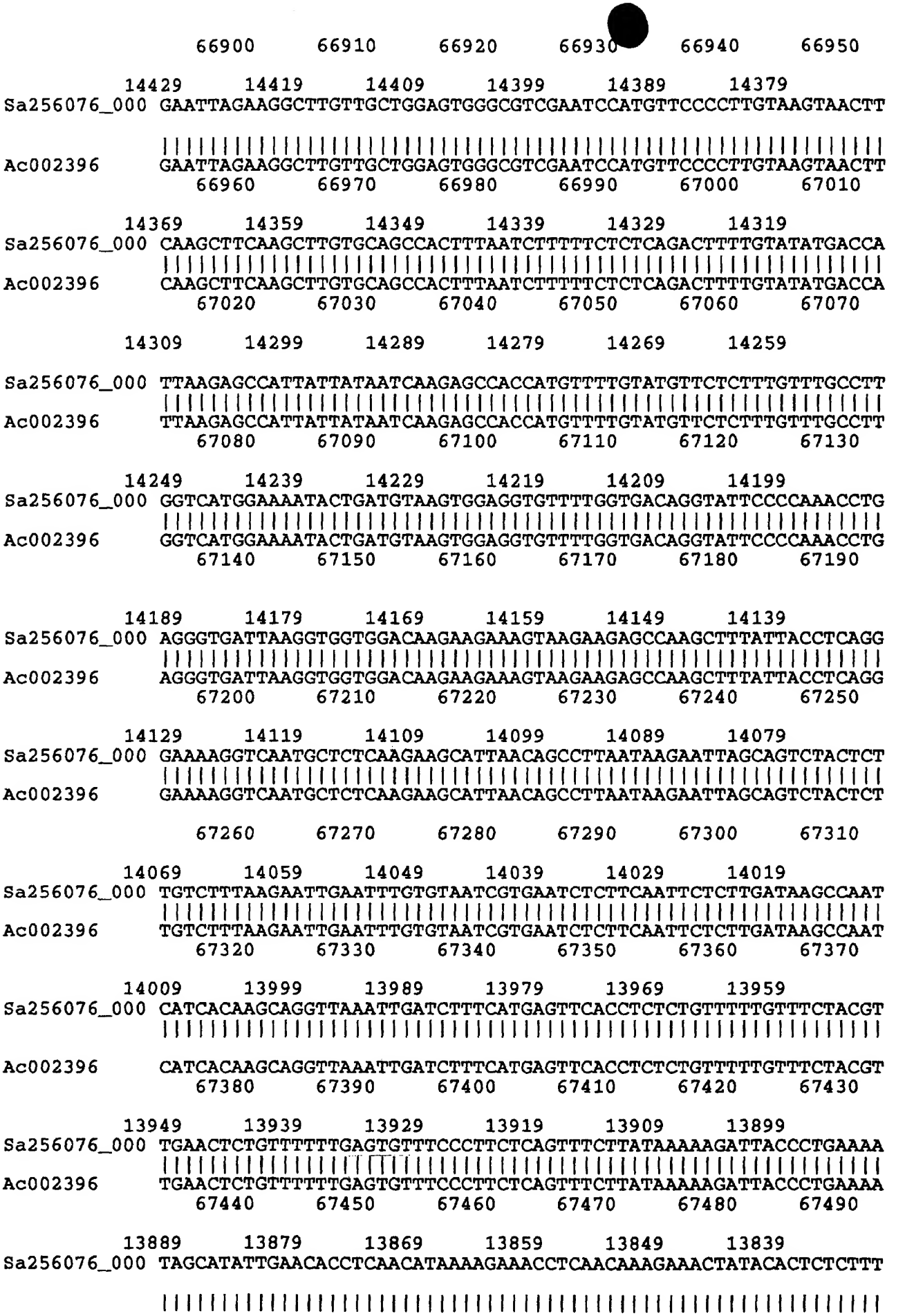

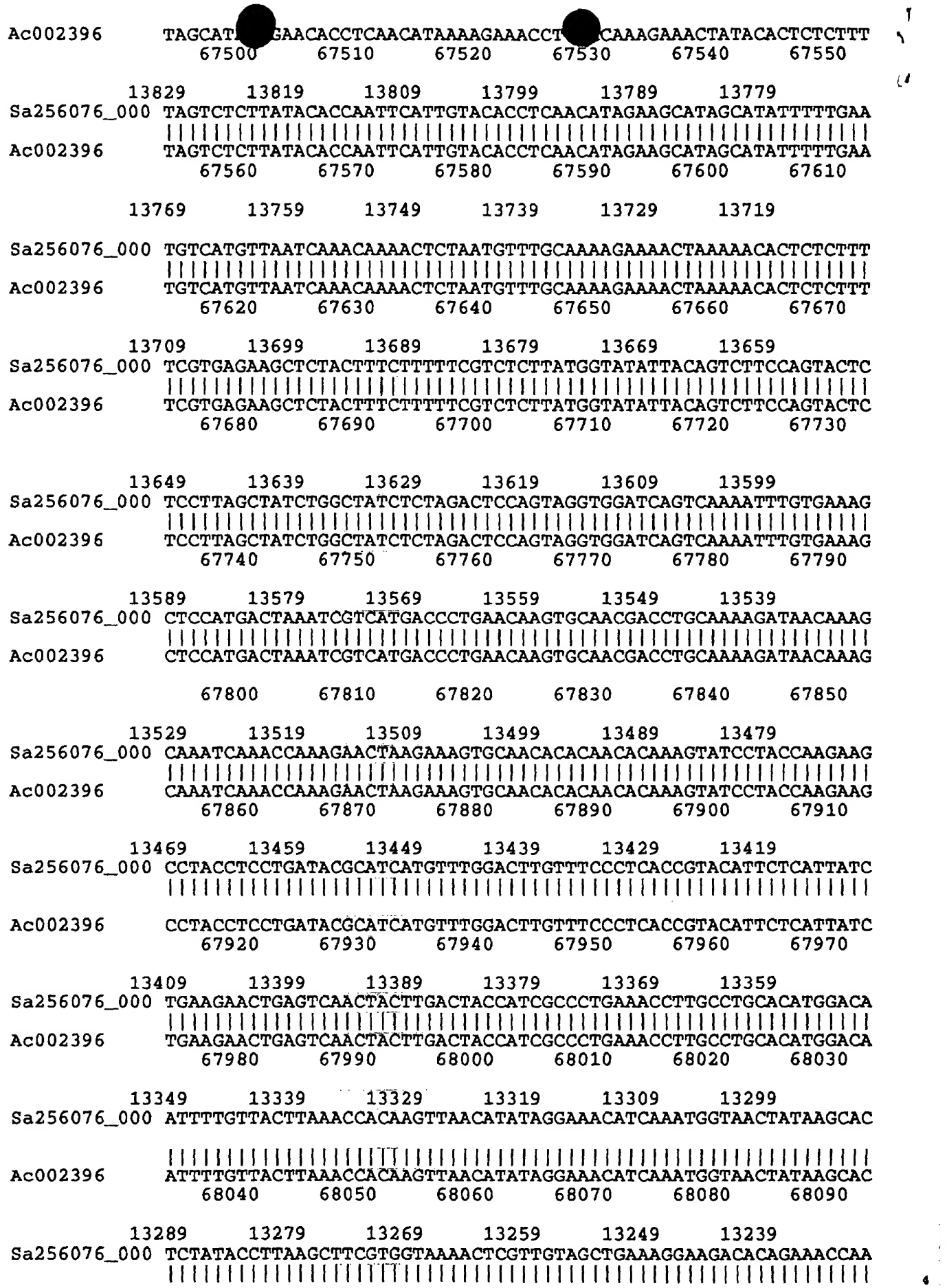

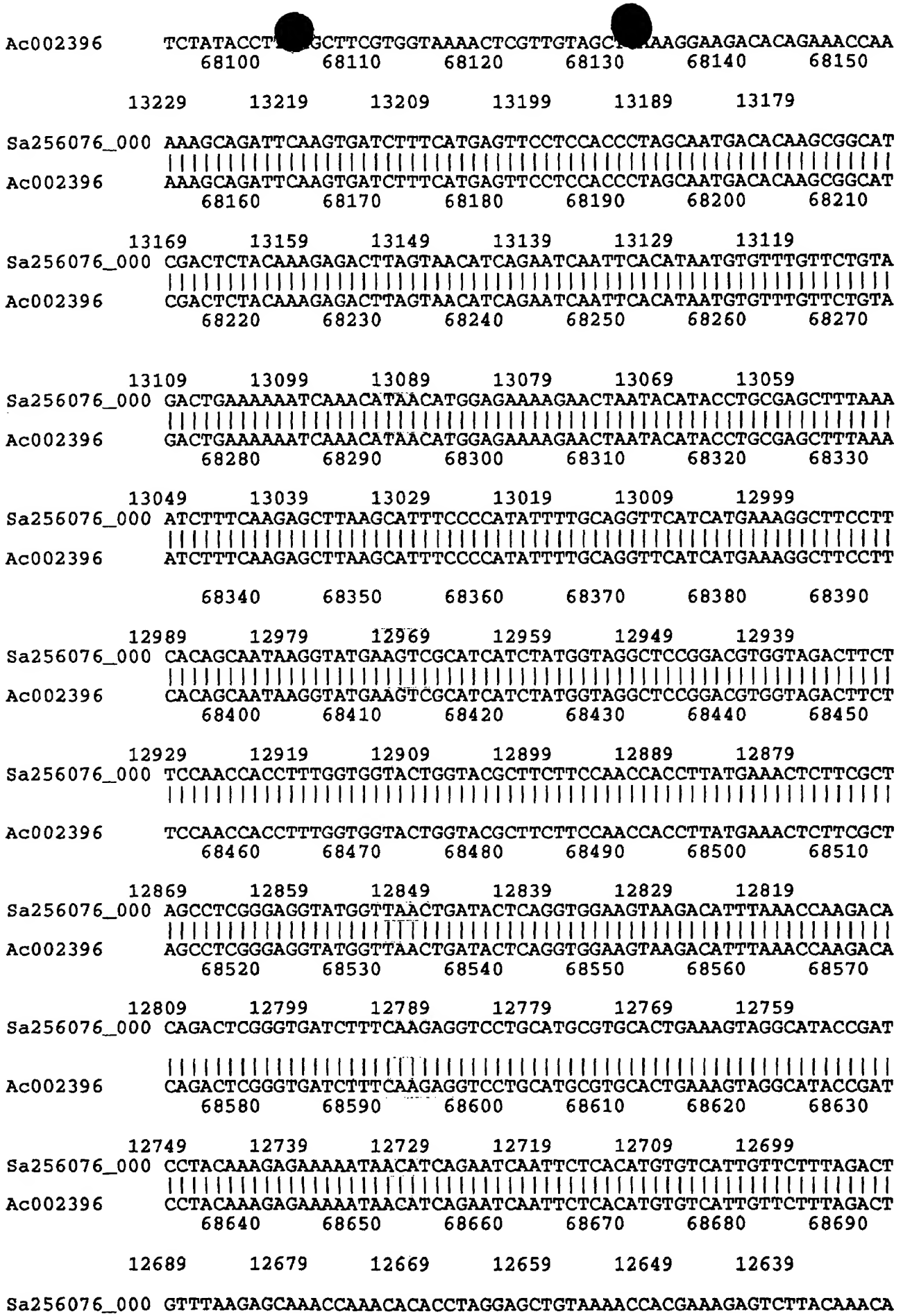

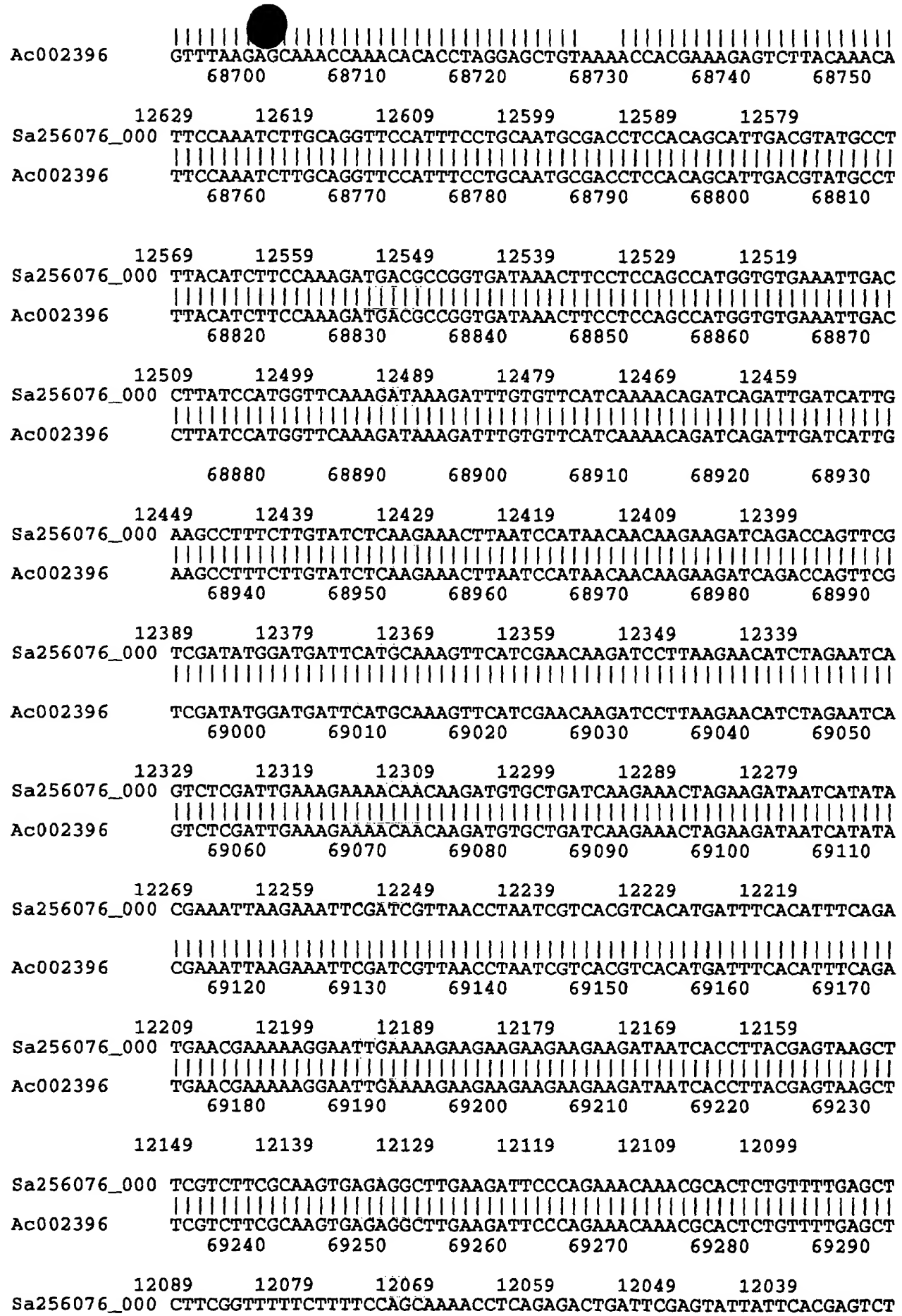

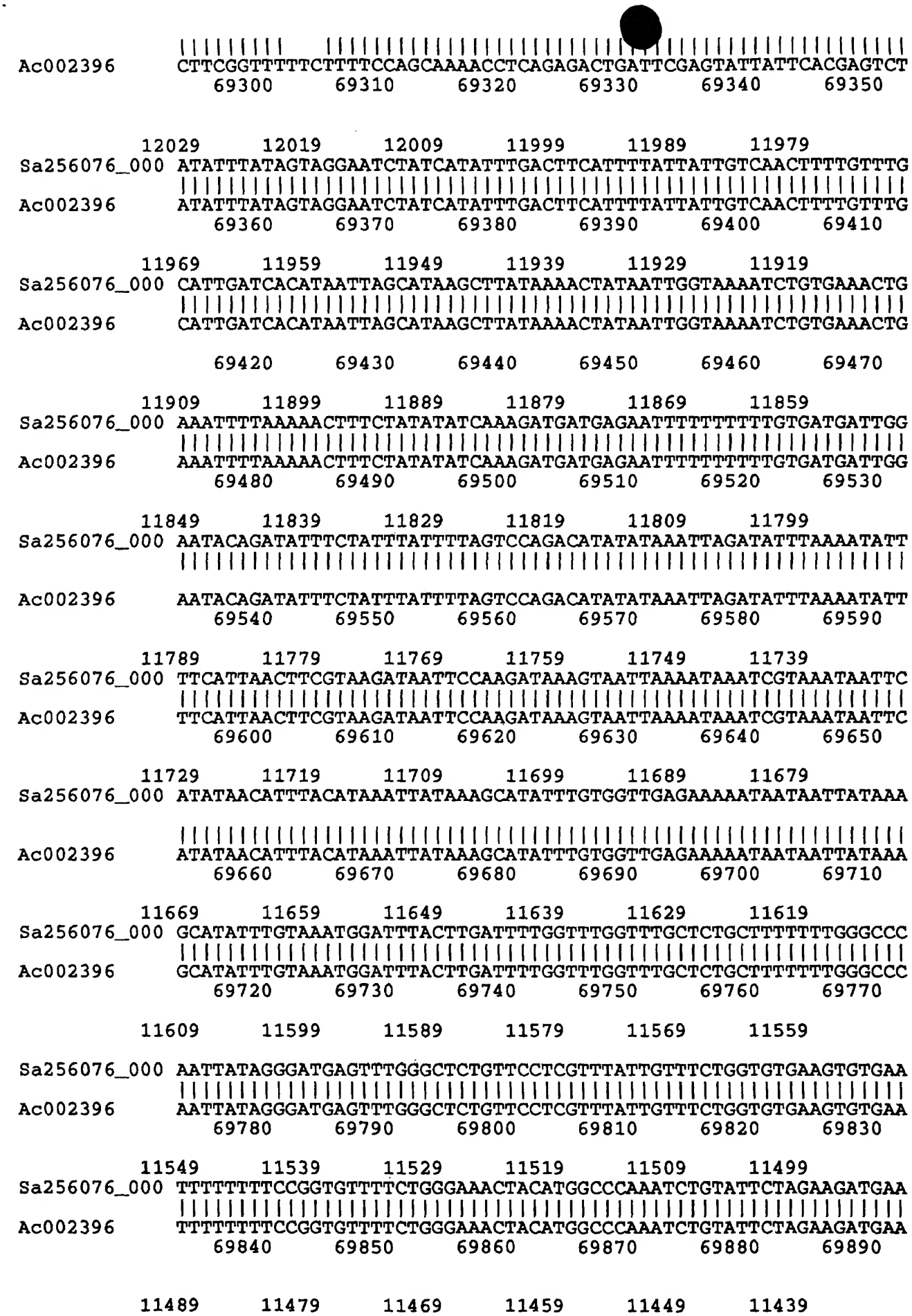

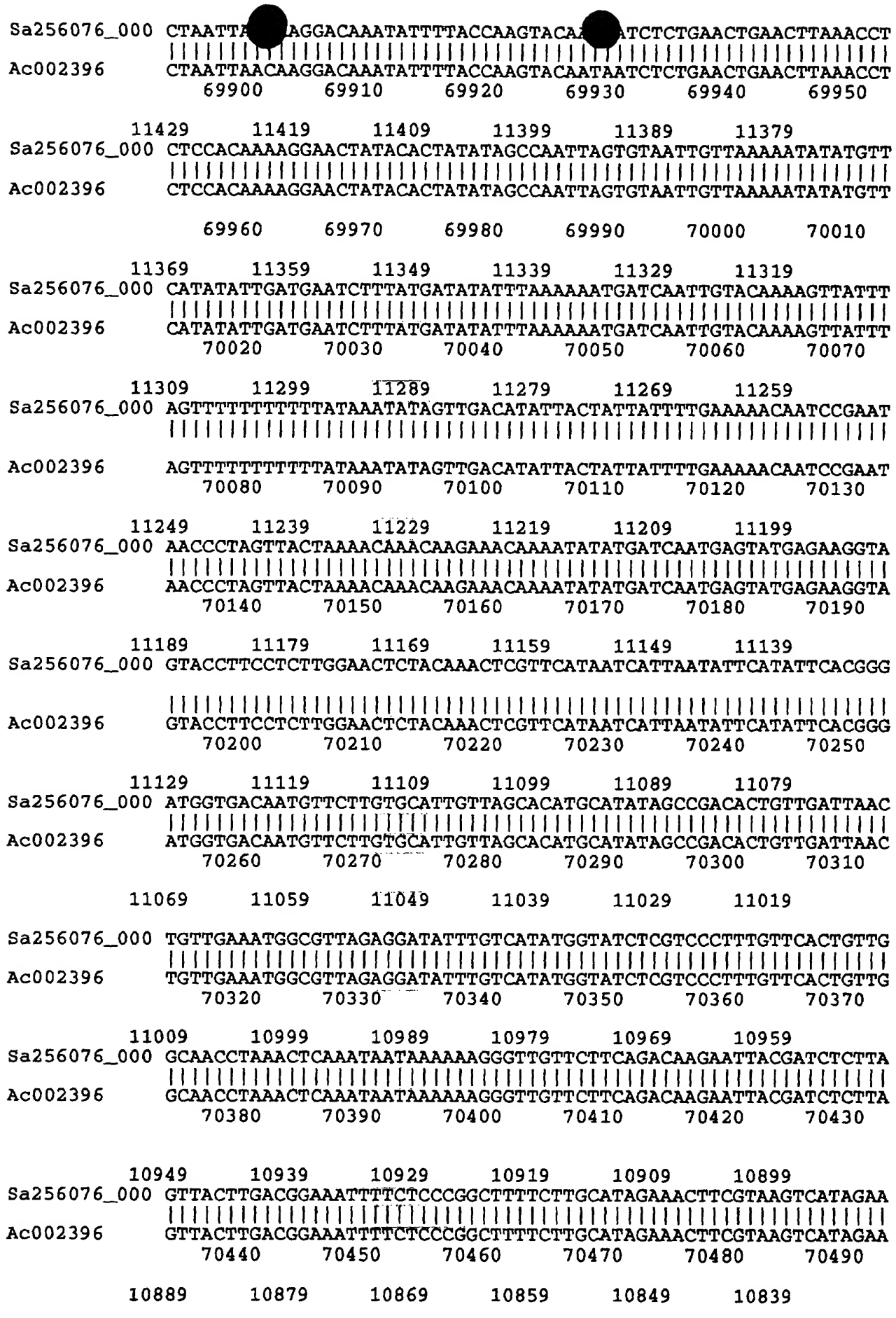

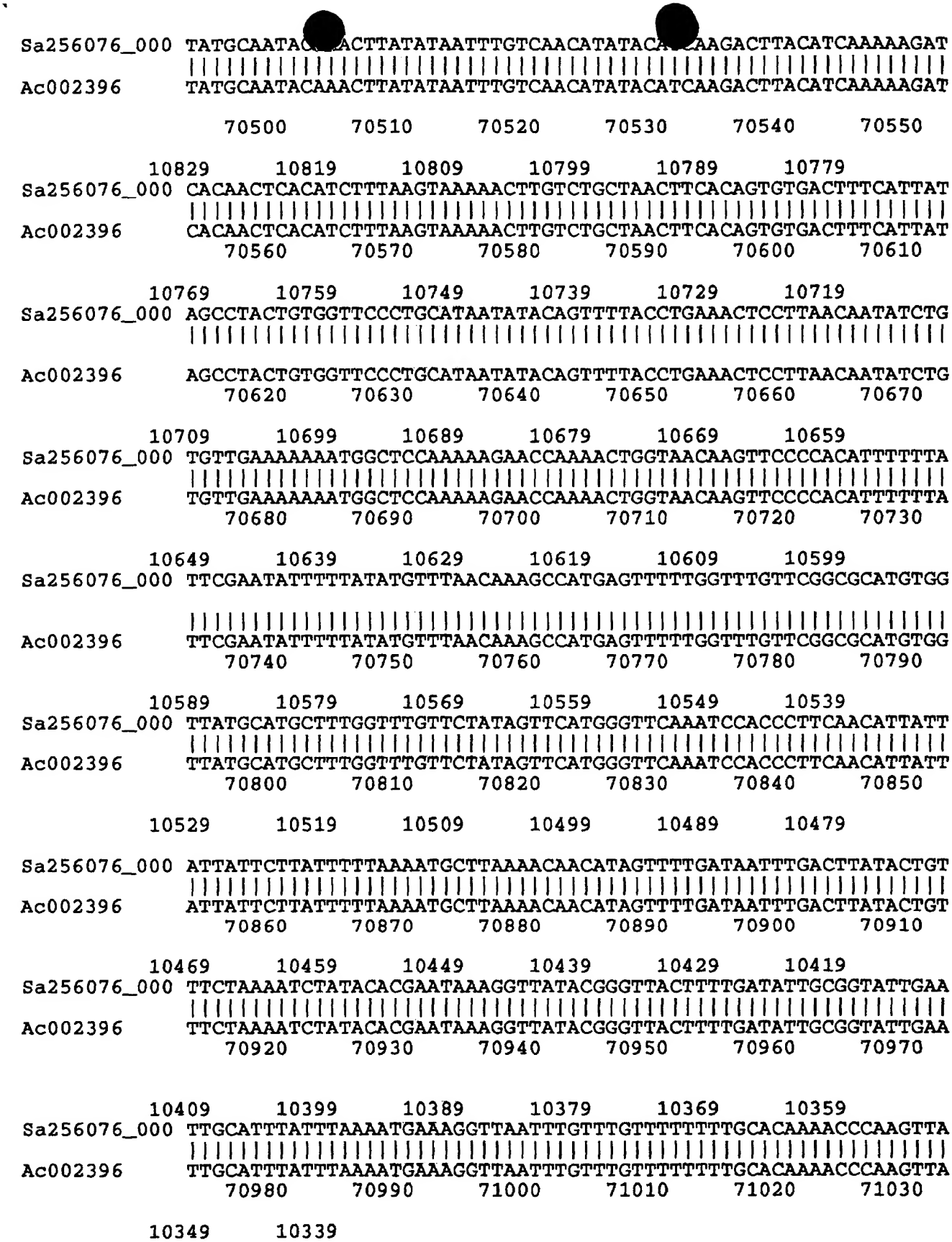

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CC   Contact: Steve Rounsley
CC   Department of Eukaryotic Genomics
CC   The Institute for Genomic Research
CC   9712 Medical Center Dr., Rockville, MD 20850, USA
CC   Tel: 301 838 0200
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AGL1–AGL6, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes

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Division of Biology, California Institute of Technology, Pasadena, California 91125 USA

The predicted products of floral homeotic genes, *AGAMOUS* (*AG*) from *Arabidopsis thaliana* and *DEFICIENS A* (*DEF A*) from *Antirrhinum majus*, have been shown previously to share strong sequence similarity with transcription factors from humans (SRF) and yeast (MCM1). The conserved sequence between these proteins is localized within a domain known to be necessary for the DNA binding and for the dimerization of SRF. We have isolated six new genes from *A. thaliana*, *AGL1–AGL6*, which also have this conserved sequence motif. On the basis of the sequence comparison between the *AG* and *AGL* genes, they can be assigned to two subfamilies of a large gene family. RNA dot blot analysis indicates that five of these genes (*AGL1*, *AGL2*, *AGL4*, *AGL5*, and *AGL6*) are preferentially expressed in flowers. In addition, in situ RNA hybridization experiments with *AGL1* and *AGL2* show that their mRNAs are detected in some floral organs but not in others. Our results suggest that these genes may act to control many steps of *Arabidopsis* floral morphogenesis. In contrast, the *AGL3* gene is expressed in vegetative tissues as well as in flowers, suggesting that it functions in a broader range of tissues. We discuss possible roles of this gene family during the evolution of flowers.

[*Key Words*: Floral-specific genes; flower development; gene family; MADS box; in situ hybridization]

Received October 30, 1990; revised version accepted December 28, 1990.

Although flower development has been described in some detail, very little is known about the molecular machinery that controls cellular differentiation in developing flowers. In recent years, the small mustard *Arabidopsis thaliana* has been used increasingly for plant molecular and genetic studies (Meyerowitz 1987, 1989), and a number of *Arabidopsis* floral homeotic mutants have been characterized (Koornneef 1987; Pruitt et al. 1987; Bowman et al. 1988, 1989; Haughn and Somerville 1988; Komaki et al. 1988; Kunst et al. 1989; Meyerowitz et al. 1989). Phenotypes of several homeotic mutants indicate that they alter floral organ identities (Komaki et al. 1988; Bowman et al. 1989; Kunst et al. 1989). One of these homeotic genes is *AGAMOUS* (*AG*). Homozygous *ag* mutant plants produce double flowers (Bowman et al. 1989; Meyerowitz et al. 1989). In the *ag* mutant flower, while four sepals and four normal petals develop in the outer two whorls, as in the wild type, six additional petals occupy the wild-type positions of stamens. In addition, a new flower appears in the position occupied in wild type by the ovary. The pattern of 4 sepals surrounding 10 (4 + 6) petals repeats until the whole flower has ~70 organs (Bowman et al. 1989). The *AG* gene has been cloned recently (Yanofsky et al. 1990), and DNA sequence analysis indicates that it encodes a protein that shares striking similarity in its amino-terminal portion with the DNA-binding domains of transcription factors from humans (SRF; Norman et al. 1988) and yeast (MCM1; Passmore et al. 1988), suggesting that the AG protein is a transcription factor. Another yeast regulatory gene, *ARG80* (Dubois et al. 1987), also has the same type of sequence motif.

Approximately a dozen genes have been defined genetically to be required for normal floral morphogenesis in *Arabidopsis* (Koornneef 1987). The complex process of flower development is likely to require many more regulatory proteins to coordinate the formation of floral organs at the proper time and location. In *Drosophila*, many of the regulatory proteins that control early developmental fate share a conserved domain for similar functions, e.g., DNA binding (Ingham 1988). By analogy, it is possible that the conserved putative DNA-binding domain of AG is shared by other regulators of flower development. In fact, a recently cloned flower homeotic gene from *Antirrhinum majus* (snapdragon), *DEF A*, also encodes a protein with the same type of DNA-binding domain (Sommer et al. 1990). This conserved motif has since been called the MADS box (for MCM1, AG and ARG80, DEF A, and SRF). Mutations in the *DEF A* gene cause phenotypes in snapdragon flowers that are very

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different from those of *ag* mutants: The petals are replaced by sepals, and the stamens are replaced by carpel-like tissues, while the outer sepals and inner carpels are normal (Sommer et al. 1990).

In an effort to gain further understanding of *Arabidopsis* flower development, we set out to isolate genes that share sequence similarity with *AG*. Here we report the isolation and characterization of six genes that share substantial sequence similarity with *AG* and *DEF A*. They are designated *AGL1–AGL6* for AG-like. We present the sequences of the *AGL* genes and their expression patterns. The possible functions of this large family of regulatory genes in flower development, and its possible role in the evolution of the flower, are discussed.

Results

Isolation of AGL *genomic and cDNA clones*

The region of amino acid sequence similarity between the AG protein (Yanofsky et al. 1990), the known transcription factors SRF (Norman et al. 1988) and MCM1 (Passmore et al. 1988), and the yeast regulatory protein ARG80 (Dubois et al. 1987) is localized within a 56-residue domain (the MADS box) in the amino-terminal region of these proteins. A highly conserved octapeptide, KKAYELSV, is found within the MADS box. A set of degenerate oligonucleotides was generated based on this octapeptide (see Materials and methods). Low-stringency hybridization of an *Arabidopsis* genomic DNA blot with this set of oligonucleotides as probes revealed ~20 bands (data not shown). These oligonucleotides were then used to probe a cosmid library (Yanofsky et al. 1990) made from *Arabidopsis* nuclear DNA, and 46 clones were isolated. Southern blot analysis showed that 12 of the clones hybridized to an *AG* cDNA clone under moderate stringency (data not shown). On the basis of patterns of restriction fragments and hybridization with the *AG* cDNA, we concluded that these 12 clones most likely represent four genes, named *AGL1–AGL4*. This was confirmed later by DNA sequencing (see below). Representative cosmids were chosen for further analysis.

AGL1 and *AGL2* genomic fragments were used to probe a λgt10-based cDNA library constructed from *Arabidopsis* floral poly(A)$^+$ RNA (Yanofsky et al. 1990). DNA sequence analysis (see below) revealed that among the cDNA clones isolated with an *AGL1* probe (probe 1, Fig. 1B) there were not only clones for *AGL1* but also for *AGL2* and for one additional gene, designated *AGL5* (Fig. 1A). Similarly, *AGL2* and *AGL4* cDNA clones (Fig. 1A) were isolated with an *AGL2* probe (probe 2, Fig. 1B). Because these clones hybridize to the *AG* gene at moderate stringencies, we probed the cDNA library with an *AG* cDNA at a moderate stringency (see Materials and methods). Only moderate to weak positives were analyzed; a total of 27 *AGL* clones, including *AGL3* and another

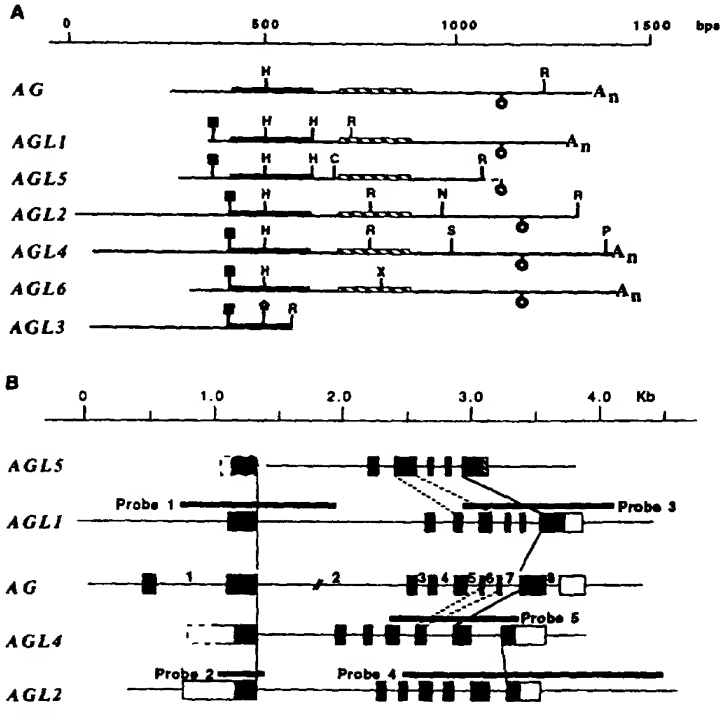

Figure 1. (*A*) Maps of *AGL* cDNAs. (*AG* and *AGL1*, *AGL2*, *AGL4*, and *AGL6*) Composite maps from two cloned fragments, the ends of which are marked by *Eco*RI sites (the cDNA and genomic sequences agree with each other; for clone numbers, see Materials and methods). All clones have *Eco*RI sites (all are not shown) at both ends; only *Eco*RI, *Hin*dIII, and other sites used for demarcation purposes are shown. The symbols (■) and (○) indicate the positions of translational initiation and termination codons, respectively. The A_n signs represent poly(A) tails. The solid bars indicate the position of the MADS boxes, and the hatched bars indicate the position of the conserved K boxes. The region at the carboxyl terminus of *AGL5*, represented by the dashed line, is from genomic sequence. The asterisk (*) in *AGL3* indicates the position of sequence AGGCTT, one base different from the *Hin*dIII site (AAGCTT) found at this position in the other cDNAs. Enzyme keys: (C) *Sca*I; (H) *Hin*dIII; (N) *Nde*I; (P) *Hpa*I; (R) *Eco*RI; (S) *Ssp*I; (X) *Xho*I. (*B*) *AGL1*, *AGL2*, *AGL4*, *AGL5* gene structures (for clone numbers, see Materials and methods). The boxes indicate exons (open boxes represent untranslated regions), and the lines between them represent introns. All of the introns have the canonical donor and acceptor sites, GT and AG, respectively. The boxes with dashed lines at the 5′-most portion of *AGL4* and *AGL5* represent regions of uncertainty because of the lack of genomic information; the hatched box at the 3′ region of *AGL5* lacks cDNA confirmation. The bars above *AGL1*, *AGL2*, and *AGL4* represent regions of the corresponding genes used as probes to isolate cDNA clones. The dashed lines indicate the introns that are lacking in some genes; the other introns between the solid lines connecting different genes have conserved positions.

gene, *AGL6* (Fig. 1A), were isolated. *AGL5* genomic cosmids (probed with an *AGL5* cDNA) and additional *AGL2* and *AGL4* cosmids (probed with an *AGL2* cDNA) were isolated from the cosmid library. The 3′ portions of *AGL1*, *AGL2*, and *AGL4* cDNAs were isolated subsequently using gene-specific genomic fragments as probes.

The AGL *gene structures and nucleotide sequences*

We have determined the sequences of *AGL* cDNAs (Figs. 2–4; *AGL3* cDNA sequence is incomplete and not shown), as well as the entire genomic regions for *AGL1* and *AGL2* and most of the *AGL4* and *AGL5* genes. On the basis of the comparison between cDNA and genomic sequences, we have deduced the complete exon–intron structures for *AGL1* and *AGL2* and nearly complete structures for *AGL4* and *AGL5* (Fig. 1B). The intron positions are largely, though not entirely, conserved in all of the genes where the intron positions are known (Fig. 1B).

The *AGL1*, *AGL4*, and *AGL6* cDNAs each contain a large open reading frame (ORF), as well as 5′- and 3′-nontranslated regions and a poly(A) tail (Figs. 1A, 2–4). Although the cDNA clones for *AGL2* and *AGL5* do not include poly(A) tails (see Materials and methods), the entire protein-coding regions for these two genes have been identified (Figs. 1A, 2, and 3). The *AGL5* cDNA clone does not contain the termination codon for the longest ORF, but comparison of the genomic sequence matching the end of the *AGL5* cDNA with the carboxy-terminal sequences of *AG* and *AGL1* suggests the probable carboxyl terminus of the AGL5 protein (Fig.2). The sequence of the amino-terminal portion (including most of the MADS box) of the AGL3 protein (Fig. 5B) has been deduced from the cDNA sequence (data not shown). Additional AGL3 protein sequence (Fig. 5B) has been deduced from genomic sequence (data not shown) using the canonical intron donor (GT) and acceptor (AG) sites. The proteins encoded by the *AGL* genes are small (28.2–28.8 kD calculated molecular mass) and slightly basic, simi-

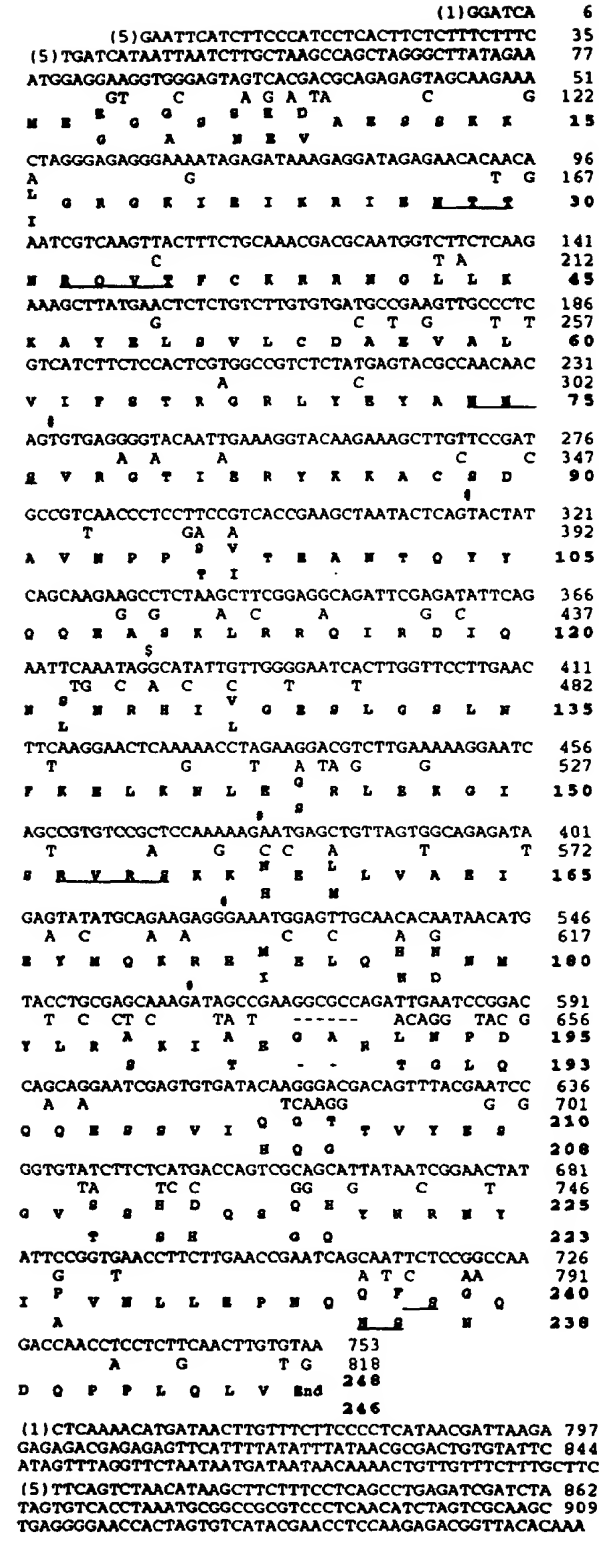

Figure 2. *AGL1* and *AGL5* cDNA and deduced protein sequences. The complete coding region of *AGL1* is shown, and only the nucleotides in *AGL5* that are different from *AGL1* are shown below the *AGL1* sequence. The amino acid sequences are shown in boldface. The dashes in both nucleotide and amino acid sequences indicate gaps introduced to allow the best alignment. Where AGL1 and AGL5 amino acid residues are identical, they are shown once; where they are different, the AGL1 residues are shown above the AGL5 residues. For *AGL5*, the DNA sequence starting at nucleotide 782 and the last 12 amino acids residues are from genomic sequences. The calculated molecular masses for AGL1 and AGL5 are 28,337 and 28,158 daltons, respectively. The potential phosphorylation sites [RXX(T/S)] and glycosylation sites [NX(T/S)] are underlined. The positions of introns shared by *AGL1* and *AGL5* are represented by the number (| sign above the *AGL1* nucleotide sequence; the one intron that is present only in *AGL1* is indicated by the dollar ($) sign. The untranslated regions for *AGL1* and *AGL5* are shown separately, as indicated.

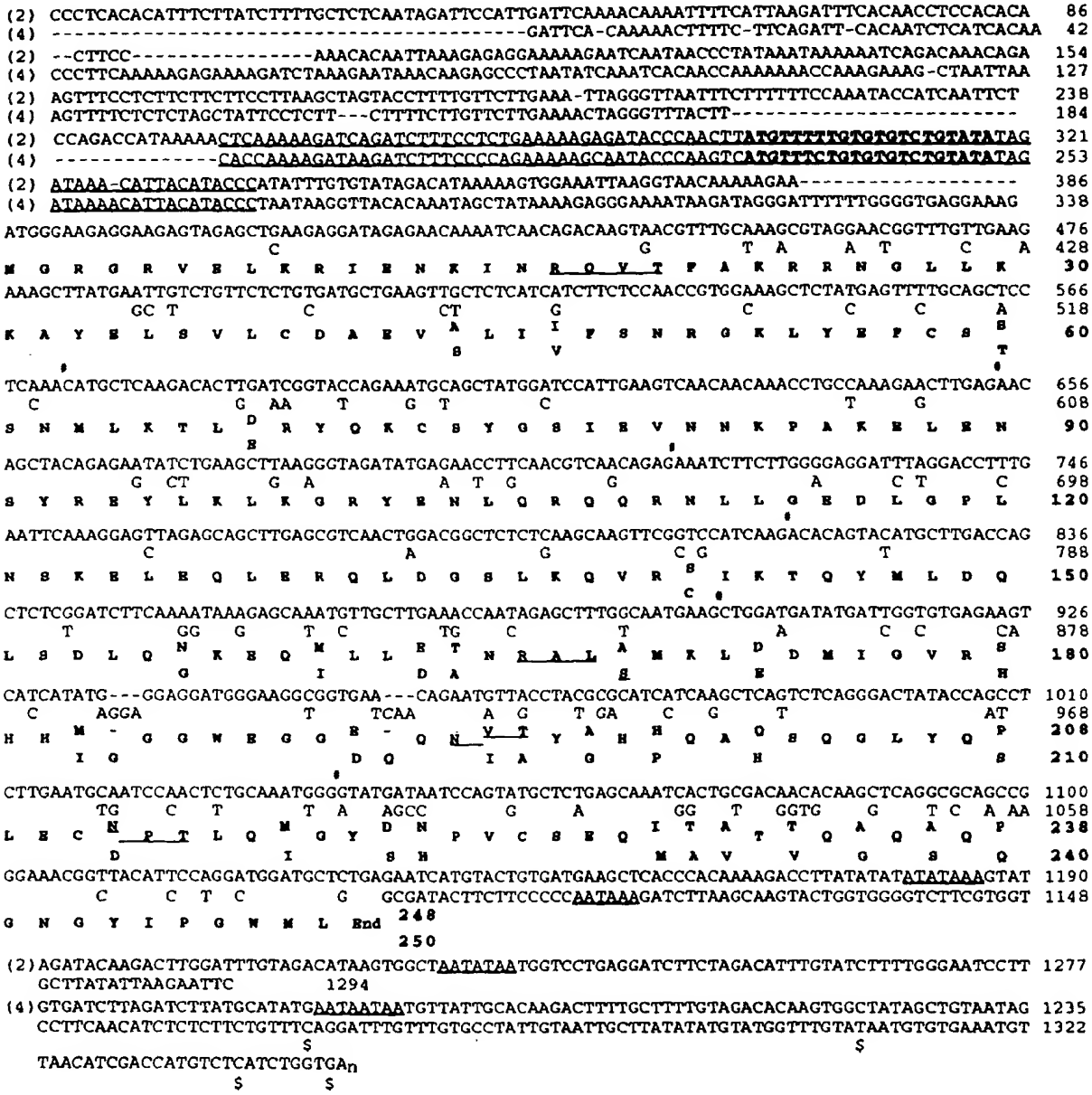

Figure 3. *AGL2* and *AGL4* cDNA and deduced protein sequences. The coding regions are shown in the same way as in Fig. 2, with *AGL2* sequences above *AGL4* sequences. The dashes in both nucleotide and amino acid sequences indicate gaps. The calculated molecular masses for AGL2 and AGL4 are 28,456 and 28,579 daltons, respectively. The 5′-untranslated regions are shown with *AGL2* above *AGL4* sequences, as indicated with (2) and (4). The two small ORFs are highlighted in boldface, and the flanking conserved sequences are underlined. The potential phosphorylation sites and glycosylation sites of the predicted proteins are underlined. Intron positions for both *AGL2* and *AGL4* are denoted with number (#) signs. The 3′-untranslated region for the two cDNAs are shown separately. In the 3′-untranslated region of *AGL4*, the dollar ($) signs indicate the positions of four observed polyadenylation sites, and potential polyadenylation signals are underlined for both *AGL2* and *AGL4*.

lar to the AG protein (Yanofsky et al. 1990). Table 1 shows the percentages of identical residues between AG and the AGL proteins in different regions. Like AG, the AGL proteins all have the MADS box, as discussed below.

The regions in the *AGL2* and *AGL4* cDNAs 5′ of the long ORFs each have a short ORF beginning with an ATG codon (Fig. 3). These short ORFs differ by only one nucleotide and potentially encode the identical heptapeptide MFLCVCI. The sequences flanking these short

```
                                                ATTTATCGTGTAC    13
GATACTTTATTCCTTTTATCTATTCTTGAAAAAAAGTTACCAATT                    58
CTTGAGAAGAAGAAGAAATCAGAATCAAGAGAAGGAGAGAGAAAG                   103
ATGGGAAGAGGGAGAGTGGAGATGAAGAGGATAGAGAACAAGATT                   148
M  G  R  G  R  V  E  M  K  R  I  E  N  K  I                      15
AATAGACAAGTGACCTTCTCAAAAAGAAGAAACGGTTTGCTGAAG                   193
N  R  Q  V  T  F  S  K  R  R  N  G  L  L  K                      30
AAAGCTTATGAGCTTTCTGTTCTTTGCGATGCCGAAGTTGCTCTC                   238
K  A  Y  E  L  S  V  L  C  D  A  E  V  A  L                      45
ATCATCTTCTCAAGCCGTGGCAAGCTCTACGAGTTTGGTAGTGTT                   283
I  I  F  S  S  R  G  K  L  Y  E  F  G  S  V                      60
GGAATTGAAAGCACAATCGAACGGTATAATCGTTGTTACAACTGC                   328
G  I  E  S  T  I  E  R  Y  N  R  C  Y  N  C                      75
TCTCTAAGCAATAATAAGCCTGAAGAGACTACACAGAGTTGGTGT                   373
S  L  S  N  N  K  P  E  E  T  T  Q  S  W  C                      90
CAGGAGGTGACAAAGCTTAAATCCAAATACGAATCTCTTGTTCGT                   418
Q  E  V  T  K  L  K  S  K  Y  E  S  L  V  R                     105
ACTAACAGGAATTTGCTTGGAGAAGATCTTGGAGAAATGGGTGTG                   463
T  N  R  N  L  L  G  E  D  L  G  E  M  G  V                     120
AAGGAACTGCAAGCGCTCGAGAGGCAGCTCGAAGCCGCTCTTACC                   508
K  E  L  Q  A  L  E  R  Q  L  E  A  A  L  T                     135
GCGACTCGACAGCGCAAGACACAAGTTATGATGGAAGAAATGGAA                   553
A  T  R  Q  R  K  T  Q  V  M  M  E  E  M  E                     150
GACCTTAGGAAAAAGGAGAGGCAACTAGGAGACATAAACAAACAA                   598
D  L  R  K  K  E  R  Q  L  G  D  I  N  K  Q                     165
CTCAAGATTAAGTTTGAAACGGAAGGCCATGCTTTCAAAACCTTT                   643
L  K  I  K  F  E  T  E  G  H  A  F  K  T  F                     180
CAAGACTTATGGGCAAACTCGGCGGCATCGGTGGCCGGGGATCCA                   688
Q  D  L  W  A  N  S  A  A  S  V  A  G  D  P                     195
AACAATTCTGAATTTCCGGTAGAGCCTTCTCATCCTAATGTATTG                   733
N  N  S  E  F  P  V  E  P  S  H  P  N  V  L                     210
GATTGCAACACCGAACCCTTTTTACAAATAGGGTTTCAACAACAT                   778
D  C  N  T  E  P  F  L  Q  I  G  F  Q  Q  H                     225
TACTACGTGCAAGGTGAAGGGTCTTCGGTATCAAAGAGTAACGTG                   823
Y  Y  V  Q  G  E  G  S  S  V  S  K  S  N  V                     240
GCAGGTGAGACTAATTTCGTCCAAGGTTGGGTTCTTTGA   862
A  G  E  T  N  F  V  Q  G  W  V  L  End  252
CTCTCTGTTGATTAGCCCACGATGCCACGGTCAGGCCAATTTCAGC   908
TCTCTACAGTTGTTCTTTTTTCAAATTAGATTTCTGGTTTTTTTTT   954
TCCTATAAGAAAAACTTTTGCACTAGATGTTTGTCATTTAATTTCC  1000
AGCTCGTGTGAATCTATATTCGCATGTATGTGCTTTGAAGAATTTC  1046
                    $
TCCTCTTACTCCTACTTGATCTAAAACATTATTTTTGTTTTGGGTTTAn
                                                    $
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Figure 4. *AGL6* cDNA and deduced protein sequence. The calculated molecular mass for AGL6 is 28,744 daltons. The potential phosphorylation sites and glycosylation sites are underlined. The positions of two observed polyadenylation sites are denoted with a dollar ($) sign below the nucleotide.

ORFs are also highly conserved between *AGL2* and *AGL4* (36/45 and 17/18 identity for 5′ and 3′, respectively). However, the nucleotides immediately adjacent to the ATGs of these small ORFs do not match the plant initiation consensus sequence: (A/T)(C/A)AACAATGGC (Lütcke et al. 1987). The presence of short ORFs upstream of the protein-coding region has been observed previously for other genes in yeast (Hinnebusch 1984; Werner et al. 1985; Forsburg and Guarente 1989), in animals (Kozak 1987), and in plants (Ma et al. 1990; Schmidt et al. 1990). In yeast, it is known that the short ORFs in the *GCN4* and *CPA1* (Hinnebusch 1988) mRNA are required for proper translational regulation.

Map positions of AGL1, AGL2, *and* AGL3

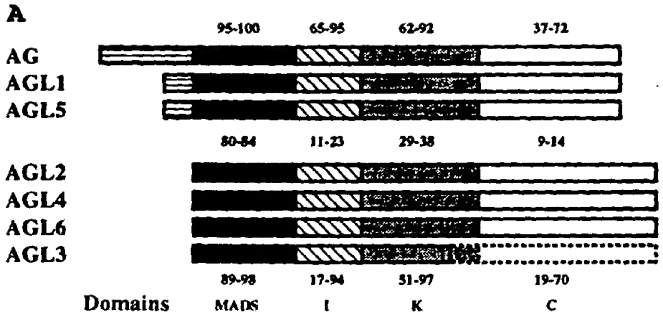
As a step toward determining whether the *AGL*s correspond to any genes identified previously by genetic analysis, we localized *AGL1*, *AGL2*, and *AGL3* relative to other molecular markers using restriction fragment length polymorphisms (RFLPs; Chang et al. 1988). *AGL1* maps on chromosome 3 near the lower end, ~0.6 cM from the marker 460 on the RFLP map constructed by Chang et al. (1988); *AGL2* maps on chromosome 5 about 0.6 cM centromere-proximal from the chalcone synthase gene; and *AGL3* maps on chromosome 2 near the upper end, ~2.6 cM from the marker 246. From these mapping results, *AGL1–3* do not appear to coincide with any gene identified previously by mutations. The *AGL4*, *AGL5*, and *AGL6* genes did not reveal any RFLPs between the ecotypes used in our crosses, and have not been mapped.

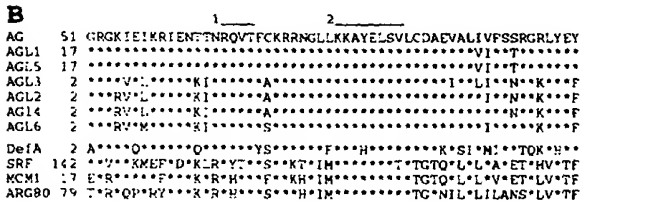
AGL1, AGL2, AGL4, AGL5, *and* AGL6 *are expressed preferentially in flowers*

Because cDNA clones for all six *AGL* genes have been isolated from a cDNA library constructed from floral poly(A)$^+$ RNA, it follows that these genes are expressed in flowers. Their expression patterns were further characterized using RNA dot blot hybridizations. RNAs from immature seed pods, flowers, stems, and leaves were spotted onto nylon filters, and identical filters were probed with each of the labeled 3′ portions of *AGL1*, *AGL2*, *AGL4*, *AGL5*, and *AGL6* cDNAs (lacking the sequences encoding the MADS box to minimize cross-hybridization) and the only available *AGL3* cDNA (including the sequences encoding the MADS box). As a contr l, radiolabeled *AG* cDNA was also used to probe one of the RNA filters. The results (Fig. 6) agree with the previous finding (Yanofsky et al. 1990) that *AG* is expressed in flowers but not in leaves or stems. Five of the *AGL* genes (except *AGL3*) are expressed preferentially in flowers, and the expression continues, albeit diminished, in immature seed pods (Fig. 6). Faint signals were also detected with *AGL1* and *AGL6* in stems, and with *AGL2* in leaves. *AGL3* is expressed in stems and leaves, as well as in flowers and seed pods (Fig. 6). As controls for cross-hybridization, in vitro transcripts of the sense orientation from *AGL1*, *AGL2*, *AGL4*, *AGL5*, and *AGL6* cDNA were synthesized and spotted on the same filter strips. No cross-hybridization between any of the *AG* and the *AGL* probes and in vitro transcripts was observed under the conditions used (Fig. 6). The approximate levels of *AGL* expression are slightly lower than that of *AG*, which was estimated to have an average abundance of 1 in 10^4 poly(A)$^+$ RNA molecules in floral tissues (Yanofsky et al. 1990). This result agrees with the observed frequency of *AGL* cDNA clones in the cDNA library.

In situ RNA hybridizations with AGL1 *and* AGL2

To determine whether these genes are expressed in an organ-specific manner, the expression patterns of *AGL1* and *AGL2* were characterized in more detail by in situ hybridization. Wild-type *A. thaliana* (Landsberg *erecta*)





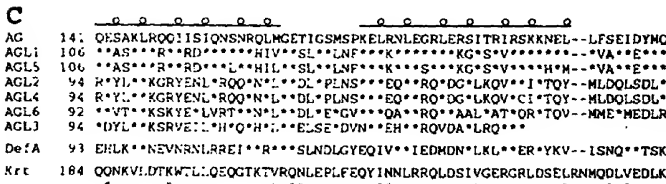


Figure 5. The comparison of deduced AG and AGL structures and alignment of conserved domains. The asterisks (*) represent identity to the first sequence of the same group; dashes indicate gaps introduced for alignment purposes. The alignment was done using the FASTP program (Lipman and Pearson 1985). (*A*) The comparison of different regions of AG and the AGL protein. The four regions (MADS, I, K, and C; see Table 1 for more detailed data on percent identities) are represented by differently shaded boxes. The numbers at the top are percent identities between AG, AGL1 and AGL5 (subfamily I); the numbers at the bottom are those between AGL2, AGL4, AGL6, and AGL3 (subfamily II); and the numbers in the middle are those between any member of subfamily I and any of subfamily II. The AGL3 information is partly derived from genomic sequence based on similarity to other AGLs and canonical intron donor and acceptor sites; the dashed-line boxes represent presumed unknown regions. The sequences in the MADS and K boxes are compared in *B* and *C*, respectively. (*B*) The alignment of the AG (Yanofsky et al. 1990) and AGL MADS boxes with those of DEF A (Sommer et al. 1990), SRF (Norman et al. 1988), MCM1 (Passmore et al. 1988) and ARG80 (Dubois et al. 1987) proteins. The conserved phosphorylation site (1) and the peptide (2) used to derive degenerate oligonucleotide sequences are indicate with lines above the AG sequence. (*C*) The alignment of the plant protein K boxes and region of the human type II keratin (Krt; see Tyner et al. 1985). Two possible helices are indicated by lines above the regions, and the circles (O) above the AG sequence indicate residues at positions a and d in the coiled coil heptapeptide repeat structure (Steinert and Roop 1988). The plus (+) signs below the keratin sequence represent an identity of the keratin residue to the corresponding residue in at least three of the AG, AGL, and DEF A sequences; the number (#) signs represent similar residues between keratin and at least four of the AG and AGL sequences.

inflorescence sections were hybridized with ^{35}S-labeled antisense RNA probes from *AGL1* and *AGL2* cDNAs. The 3′ portions of the cDNAs lacking the putative DNA-binding domain were used to avoid cross-hybridization. As shown in Figure 7, *AGL1* is expressed in carpels, particularly in ovules but not in stamens, petals, or sepals. *AGL2* is expressed mainly in carpels; in addition, the *AGL2* probe also detects a weak signal in stamens. Within the stamens, the *AGL2* signal is restricted to the anthers and is not observed in the filaments. Similar to *AGL1*, the *AGL2* signal in carpels is concentrated in ovules. The expression of *AGL1* and *AGL2*, as detected by in situ hybridization, begins in stage 10 flowers (Smyth et al. 1990) after all of the floral organs are recognizable and the ovules are visible. This onset of *AGL1* and *AGL2* expression is much later than that of *AG*, which is seen before the separation of petal and stamen primordia from the central floral primordium (G. Drews, J. Bowman, and E.M. Meyerowitz, unpubl.), in stage 3 flowers (Smyth et al. 1990). The expression of *AG*, *AGL1*, and *AGL2* all extend into later stages of flower development, including immature seed pods (Figs. 6 and 7). For *AGL1* and *AGL2*, the in situ signals are str nger in older organs (Fig. 7).

Discussion

AG *and the* AGLs *constitute a gene family*

We have identified and characterized six genes from *A. thaliana*, designated *AGL1–AGL6*. The deduced AGL proteins all share striking sequence similarity (Fig. 5) with each other and with the products of the floral homeotic genes *AG* and *DEF A*. Sequence analysis indicates that *AG* and *AGLs* are members of a diverse gene family. Table 1 shows the percentage of amino acid sequence identity between these deduced proteins in four regions. The most conserved region, the MADS box (M), is located either at or very near the amino terminus in the AGLs. The second conserved domain (the K box; see below), not found in SRF and MCM1, is near the center of the proteins, ~35 residues from the MADS box. On the basis of sequence comparison, *AG*, *AGL1*, and *AGL5* can be assigned to one subfamily, and *AGL2*, *AGL4*, and *AGL6* can be assigned to another subfamily. It is worth noting that the sequence similarity shared between members of the same subfamily is not restricted to the two conserved regions but extends throughout the entire length of the proteins (Fig. 5; Table 1). The subfamily assignment is also supported by the exon–intron struc-

Table 1. *Percentage of amino acid identity in different domains of AG, the AGLs, and DEF A proteins*

	AG		AGL1		AGL5		AGL2		AGL4		AGL3		AGL6		DEF A	
Genes	I	C	M	K	M	K	M	K	M	K	M	K	M	K	M	K
AG			**95**	68	**95**	62	**82**	32	**84**	30	**80**	37	**84**	38	**71**	24
AGL1	71	39			**100**	92	**82**	33	**80**	33	**82**	29	**82**	33	**68**	20
AGL5	65	37	95	72			**82**	36	**80**	35	**82**	33	**82**	33	**68**	23
AGL2	14	14	11	11	11	10			**98**	97	**95**	59	**95**	53	**62**	29
AGL4	17	14	14	10	14	9	94	70			**93**	57	**93**	55	**62**	29
AGL3	14	—	14	—	14	—	42	—	39	—			**89**	51	**61**	24
AGL6	23	10	23	12	23	11	34	19	29	19	17	—			**57**	23
	I	C	I	C	I	C	I	C	I	C	I	C				

The percentages for the MADS box (M, shown in boldface, 56 residues) and the second conserved domain (K, 66 residues except for AGL3, of which only 49 residues are known) are shown above the diagonal (blank space); the percentages for the sequences between the two conserved domains (I, 34–36 residues) and the carboxy-terminal regions (C, 78–98 residues) are shown below the diagonal. Because the AGL3 carboxy-terminal sequence is not known, the percentage of identity could not be calculated. See Fig. 5A for the domain organization of AG and the AGLs. The percentages of the two conserved domains (M and K) for *Antirrhinum* protein DEF A are also shown.

tures. An analysis of third-base silent changes in the MADS box indicates that the two most similar pairs (AGL1 and AGL5; AGL2 and AGL4) have much smaller percentages of difference (<30%) than other pairs, supporting the subfamily structure. Although the incomplete *AGL3* sequence does not allow definitive assignment of the *AGL3* gene, the partial sequence data indicate it is more similar to the *AGL2, AGL4,* and *AGL6* subfamily than the *AG, AGL1,* and *AGL5* subfamily. Figure 8 illustrates the relationship between AGLs and AG based on the sequence information. Sequence comparison of the *DEF A* gene to *AG* and the *AGL* genes suggests that *DEF A* does not belong to either of the two subfamilies (Table 1; Fig. 8). Low-stringency hybridization of *Arabidopsis* genomic DNA with degenerate oligonucleotides revealed ~20 bands, more than accounted for by the seven genes that have been isolated; therefore, it is likely that there are additional members of this gene family in *Arabidopsis*. We propose that members of this gene family are derived from a single ancestral gene and have arisen by gene duplication and subsequent sequence divergence and intron loss (Fig. 8).

AGLs *likely encode transcription factors*

The deduced AG and AGL amino acid sequences, as well as the product of the snapdragon floral homeotic gene *DEF A* (Sommer et al. 1990), contain a sequence motif of ~56 amino acids (the MADS box; see Fig. 5B) that is also found in the transcription factors SRF (Norman et al. 1988) and MCM1 (Passmore et al. 1988). A region of ~90 residues containing the MADS box is known to be sufficient for DNA binding and is involved in dimerization of SRF (Hayes et al. 1987; Norman et al. 1988). Recent evidence (Tan and Richmond 1990) suggests that the yeast MCM1 MADS box is also sufficient for specific DNA binding. The human SRF is involved in the regulation of the proto-oncogene c-*fos* (Treisman 1986, 1987) and a sarcomeric actin gene (Boxer et al. 1989). The yeast MCM1 gene product (GRM/PRTF) regulates mating type-specific gene expression (Herskowitz 1990). The phenotypes of *Arabidopsis ag* mutants (Bowman et al. 1989) and *Antirrhinum defA* mutants (Sommer et al. 1990) suggest that these genes play regulatory roles in specifying the identity of floral organs. The fact that the AGL proteins also contain the MADS box suggests that they are also transcription factors, possibly regulating floral morphogenesis. The AGL proteins may function in different floral cells, controlling branches of the floral morphogenesis regulatory hierarchy. Alternatively, they may function at different times, directing different stages of flower development. Although all of the AGL proteins are probably transcription factors, their expression patterns (see below) suggest they control different sets of genes.

In addition to the MADS box, the AG and AGL proteins share a second domain, which has a low but significant similarity to a portion of keratin sequences (the K

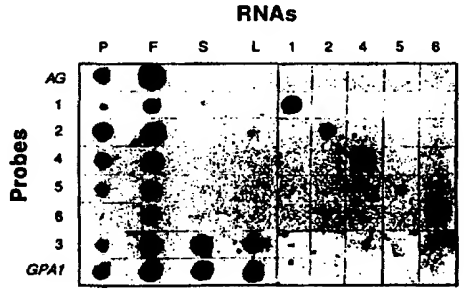


Figure 6. RNA dot blot autoradiogram. RNAs from immature seed pods (P), floral buds (F), stems (S), and leaves (L), as well as in vitro-synthesized RNAs from *AGL1* (*1*), *AGL2* (*2*), *AGL4* (*4*), *AGL5* (*5*), and *AGL6* (*6*), were spotted onto eight nylon filter strips. Each strip was hybridized with one ^{32}P-labeled cDNA: *AG*, *AGL1* (*1*), *AGL2* (*2*), *AGL4* (*4*), *AGL5* (*5*), *AGL6* (*6*), *AGL3* (*3*), and *GPA1*. *GPA1* is expressed in both floral and vegetative tissues (Ma et al. 1990). Similar amounts of radioactivity were used in all of the hybridizations. A single autoradiographic exposure was used.

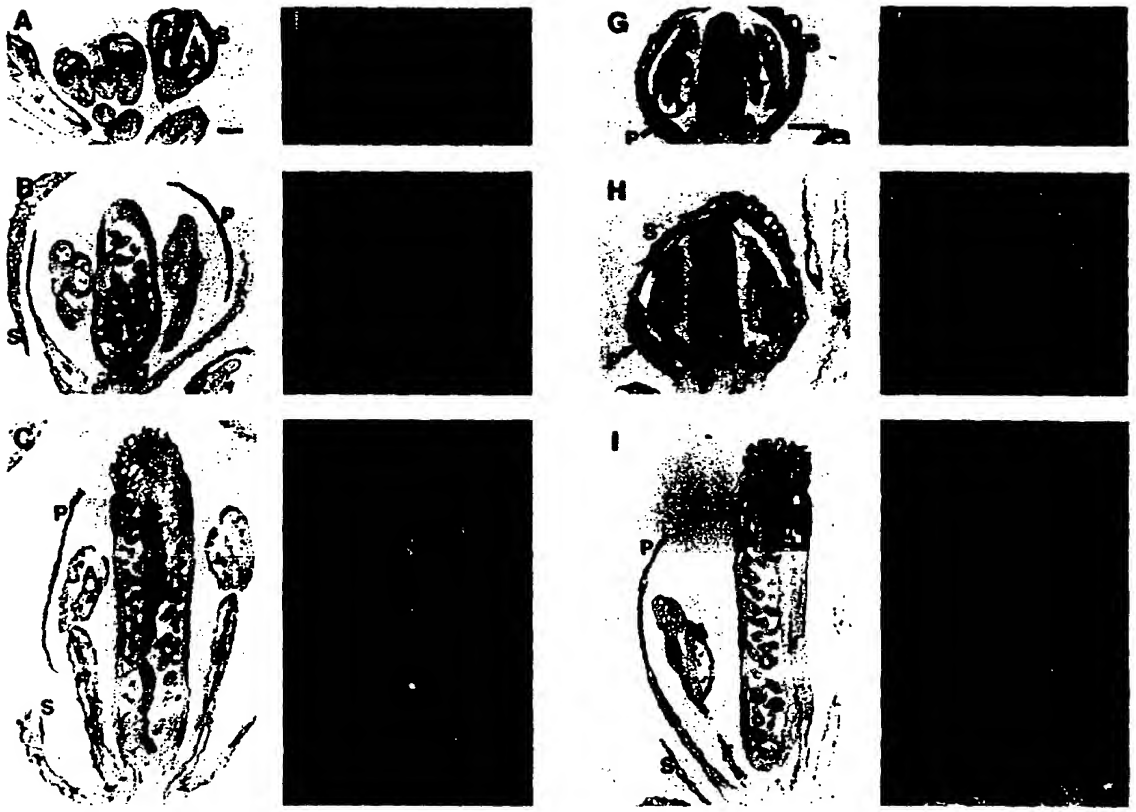


Figure 7. RNA in situ analysis with *AGL1* (*A–F*) and *AGL2* (*G–L*). *A–C* and *G–I* were photographed in bright field, and the others in dark field. For each gene, at least three developmental stages (for a description of stages 1–12, see Smyth et al. 1990) are shown: (*A* and *D*) stages 5–8; (*G* and *J*) stage 9; (*B* and *E*) stage 12; (*H* and *K*) stage 10; (*C*, *F*, *I*, and *L*) after flower opens but just before pollination; stage 13. Floral organ designations: (S) sepal; (P) petal; (T) stamen; (A) anther; (F) filament; (C) carpel; (O) ovule. The grains seen on the surface of some sepals and around the pollen grains are also seen when the sense-strand probes were used and, therefore, probably represent nonspecific sticking of the probes. Prints of one enlargement were used for *A–F*, *I*, and *L*, and a different enlargement for *G*, *H*, *J*, and *K*. (*A* and *G*) Bars, 100 μm.

box; Fig. 5C). Keratins are major components of intermediate filaments (Steinert and Roop 1988). It is known that the region of keratin with similarity to AG and AGL proteins is part of the coiled coil sequence that forms the central rod-shaped domain of keratin (Fig. 5C). The AGL sequences in this domain can potentially form two amphipathic helices.

Phosphorylation and glycosylation may modulate the activity of AGLs

SRF (Prywes et al. 1988; Ryan et al. 1989) is known to be phosphorylated. Furthermore, the phosphorylation of SRF has been shown to affect its activity (Prywes et al. 1988). Other transcription factors have been suggested to be regulated by phosphorylation, such as the yeast heat shock factor (Sorger and Pelham 1988) and GAL4 protein (Mylin et al. 1989). The difference between the apparent size (Tan and Richmond 1990) and sequence-derived size (Passmore et al. 1988) of the MCM1 protein suggests that it is also post-translationally modified. There is a conserved potential site (RQVT; Fig. 5C) for calmodulin-dependent phosphorylation (RXX(S/T); see Cohen 1988) in all of the AGLs, and most have additional sites as well (Figs. 2–4). Furthermore, it was reported that SRF is a glycoprotein (Schröter et al. 1990). Several other eukaryotic transcription factors are also glycosylated (Jackson and Tjian 1988; Lichtsteiner and Schibler 1989). The AGL proteins all have potential glycosylation sites (NXT or NXS; see Fishleigh et al. 1987; Figs. 2–4). The presence of these sites suggests that the activity of AGLs may be modulated by phosphorylation and/or glycosylation, perhaps in response to environmental and developmental signals.

Expression and functional implications of AGLs

Five of the *AGLs* are expressed preferentially in flowers and young seed pods but not (or at low levels) in leaves or stems. At this level, they are similar to known floral

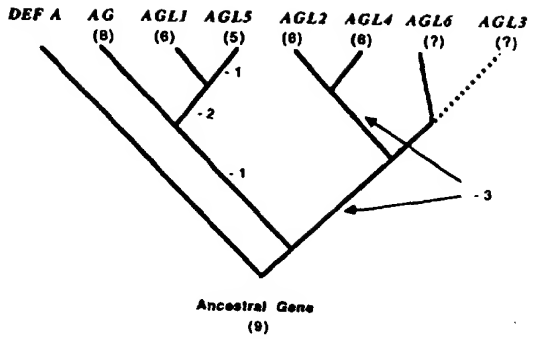


Figure 8. A proposed relationship between *AG*, *AGL* genes, and *DEF A*. The number of introns is indicated in parenthesis below each gene where it is known. The dashed line leading to *AGL3* represents the uncertainty of the position of *AGL3* due to the incompleteness of its sequence information. The time at which introns were lost during the evolution of *AGL2–AGL4*,and *AGL6* cannot be deduced because intron information is not available for *AGL3* and *AGL6*.

homeotic genes, *AG* from *A. thaliana* and *DEF A* from *A. majus*. The in situ hybridization results, on the other hand, indicate that the patterns of *AGL1* and *AGL2* expression within the flower are slightly different from that of *AG*. The *AG* expression begins in morphologically undifferentiated cells (G. Drews, J. Bowman, and E.M. Meyerowitz, unpubl.) in early developing flowers at stage 3 (Smyth et al. 1990). Later in development, *AG* is expressed in both carpels and stamens, including anthers and filaments (G. Drews, J. Bowman, and E.M. Meyerowitz, unpubl.). The onset of *AGL1* and *AGL2* expression is at stage 10, much later than that of *AG*. In addition, *AGL1* is expressed preferentially in carpels, not in stamens, petals, or sepals. The *AGL2* signal is found primarily in carpels and is lower in stamens. In carpels, the expression of both *AGL1* and *AGL2* is concentrated in ovules. Although *AGL2* and *AG* are both expressed in the stamens, *AGL2* mRNA is found only in the anthers, not in the filaments. These expression patterns suggest that *AGL1* and *AGL2* may regulate different genes from those that are controlled by *AG*. The fact that AGL1 and AGL2 share amino acid similarity with transcription factors, and that they are expressed in ovules suggest that they are both involved in regulating ovule development. The flower-specific expression of the *AG*, *AGL*, and *DEF A* genes suggests that the MADS box has been used repeatedly for flower development. On the other hand, because *AGL3* RNA is expressed in vegetative tissues as well as floral tissues, it is likely that this class of transcription factors is not exclusive to flower development.

Flowering plants appeared suddenly and very recently in evolution at ~140 million years ago (Lower Cretaceous). Some have suggested that floral organs are modified leaves, through a series of structural and functional changes during evolution (Stebbins 1976). In fact, the combination of mutations in three *Arabidopsis* homeotic genes, *AG*, *APETALA2*, and *APETALA3*, leads to "flowers" consisting of only leaf-like organs (J. Bowman, D.R. Smyth and E.M. Meyerowitz, in press). The presence of MADS-box containing genes in yeast, plants, and humans argues that this class of genes predates flowering plants. Therefore, it is likely that MADS-box regulatory gene or genes was present and functioning when flowers arose. Furthermore, during the evolution of flower structures, it is possible that these genes were duplicated, and some members diverged to take on new functions in floral morphogenesis, either interacting with different proteins, or binding to DNA with slightly different specificities. The family of genes described here includes at least one member (*AGL3*) that is expressed in both vegetative and floral tissues, presumably fulfilling a more widespread function. Additional members (*AG*, *AGL1*, *AGL2*, *AGL4*, *AGL5*, and *AGL6* and possibly others) of this gene family, presumably arisen by gene duplications, and have evolved to be preferentially expressed in flowers. At least two members of this gene family (*AG* from *Arabidopsis* and *DEF A* from *Antirrhinum*) are known to control flower development based on genetic analyses (Bowman et al. 1989; Sommer et al. 1990). Future analyses are required to test our hypotheses about the function of the other MADS box genes.

Materials and methods

Library screening, clones, and subclones

A genomic cosmid library (Yanofsky et al. 1990) made from nuclear DNA of *A. thaliana* (Landsberg *erecta*) was screened with radiolabeled degenerate oligonucleotides according to previously published procedures (Bürglin et al. 1989). The oligonucleotides 5′-ACNGANAGYTCUTANGCYTTYTT-3′(N = A, G, C or T; U = A or G; Y = C or T) are based on the conserved heptapeptide KKAYELSV in the MADS box (Yanofsky et al. 1990). More than 70 positives were detected among colonies of four genomes worth, and cosmid DNA of 46 of the positives were purified. Additional screening of the cosmid library with *AGL2* and *AGL5* cDNAs was done as described previously for hybridization of genomic DNA (Chang et al. 1988). Representative cosmids were characterized further: *AGL1*, pCIT1202 and pCIT1210; *AGL2*, pCIT1243; *AGL3*, pCIT1216; *AGL4*, pCIT1247 and pCIT4244; and *AGL5*, pCIT4243. *AGL6* cosmids have not yet been isolated. Portions of the cosmids pCIT1202, 1243, 4244, and 4243 are shown in Figure 1B.

About 1×10^6 plaques of a cDNA library constructed from floral bud poly(A)$^+$ RNA (Yanofsky et al. 1990) were screened at a moderate stringency (as described previously by Chang et al. 1988, except that the hybridization and washes were done with 5× SSPE at 52°C) with a 977-bp *AG* cDNA *Eco*RI fragment (pCIT565) as a probe. The cDNA library was also screened at high stringency (65°C hybridization and a final wash with 0.2× SSPE) with several probes (Fig. 1B): probe 1, a 1.1-kb *Dra*I *AGL1* genomic fragment (from pCIT1202); probe 2, a 0.36-kb *Bgl*II *AGL2* genomic fragment (from pCIT1243). During the construction of the cDNA library, cDNAs with internal *Eco*RI site(s) were cleaved and then ligated into separate vector molecules. The *AGL1*, *AGL2*, *AGL3*, *AGL4*, and *AGL5* cDNAs all contain at least one *Eco*RI site, therefore, each of the cDNA had to be isolated as two or more separate fragments. The 3′ portions of *AGL3* and *AGL5* cDNAs have not yet been isolated. The portions encoding the amino terminus containing the conserved DNA-binding domain (Fig. 1A) were isolated first. The portions

of the cDNAs encoding the carboxy-terminal half for *AGL1*, *AGL2*, and *AGL4* (Fig. 1A) were isolated subsequently using the respective gene-specific genomic probes (probes 3–5, Fig. 1B): probe 3 (*AGL1*), a 0.41-kb *EcoRI–BglII* and 0.79-kb *BglII* fragments (from pCIT1202); probe 4 (*AGL2*), a 2.0-kb *HindIII* fragment (from pCIT1243); and probe 5 (*AGL4*), a 1.0-kb *EcoRI–BglII* fragment (from pCIT4244). The *AGL1*, *AGL2*, and *AGL4* genomic sequences were determined, and each agrees with the corresponding cDNA sequences on both sides of the *EcoRI* sites. Furthermore, additional *AGL6* cDNA clones were isolated using the first cDNA clone (pCIT3209) as a probe. The following cDNAs are shown in Figure 1A: *AGL1*, pCIT2241(5′) and 4219 (3′); *AGL2*, pCIT3228 (5′) and 4221 (3′); *AGL3*, pCIT2280 (5′); *AGL4*, pCIT3227 (5′) and 4233 (3′); *AGL5*, pCIT2242 (5′); and *AGL6*, pCIT3209. In addition, pCIT3216 is identical to pCIT2242; pCIT2299 (lacking the MADS box) is a subclone of pCIT2242 containing a region 3′ of the *ScaI* site (Fig. 1A); pCIT4210 (lacking the MADS box) contains a portion of an *AGL6* cDNA 3′ of the *XhoI* site (Fig. 1A); and pCIT4214 is the same as pCIT4233 except at the 3′ end where pCIT4214 lack 23 nucleotides and the poly(A) tail.

DNA sequencing and RFLP analysis

Genomic and cDNA fragments were subcloned into pGEM3Zf(+) and pGEM7Zf(+) vectors (Promega) for sequencing. Sequencing was done using the Sequenase Kit (U.S. Biochemical) according to the provided protocol. Both strands were sequenced, unless otherwise noted.

RFLP mapping was done as described by Chang et al. (1988). For *AGL1*, a 1.8-kb *BglII* fragment from cosmid clone pCIT1210 was found to reveal a *BglII* polymorphism between the Columbia and Niederzenz ecotypes of *Arabidopsis*, and it was used to probe filters carrying DNAs from one of the crosses used to generate a RFLP map (Chang et al. 1988). Similarly, a ~13-kb *EcoRI* fragment from *AGL2* cosmid pCIT1243 revealed an *EcoRI* polymorphism between Columbia and Niederzenz ecotypes and was used to probe filters from the same cross. Additional hybridization of filters with DNAs from a cross between Columbia and Niederzenz (*XbaI* polymorphism) and a cross between Landsberg and Niederzenz (*BglII* polymorphism; Chang et al. 1988) were performed using an *AGL2 BglII–EcoRI* fragment (subclone pCIT1273 from cosmid pCIT1243) as a probe. For *AGL3*, a subclone (pCIT1291)with a ~7-kb *BglII* fragment from cosmid pCIT1216 uncovered an *XbaI* polymorphism between Columbia and Niederzenz ecotypes and a *BglII* polymorphism between Landsberg and Niederzenz ecotypes and was used to probe appropriate filters. The data from DNA blot hybridization experiments were analyzed using the MAPMAKER computer program (Lander et al. 1987) to obtain linkage information with respect to existing markers on the RFLP map (Chang et al. 1988).

RNA analyses

Poly(A)$^+$ RNA was isolated from developing seed pods (3–5 days after pollination), floral buds (stages 1–12, Smyth et al. 1990), floral stems, and leaves, according to procedures described previously (Crawford et al. 1986). For RNA dot blot analysis, 15 μg of total RNA from each of the four tissues was spotted onto a nylon filter (Hybond N, Amersham), and hybridized with *AG* and *AGL* cDNAs labeled with ^{32}P using random priming methods. In addition, one filter was probed with labeled cDNA of the *GPA1* gene, which is expressed in stems and leaves, as well as in flowers (Ma et al. 1990). The following plasmids (see Fig. 1A; unless otherwise noted, the entire insert was used) were used for probe synthesis: pCIT565 (*AG*, with putative DNA binding domain), pCIT4219 (*AGL1*), pCIT4221 (*AGL2*, 3′ *NdeI–EcoRI* fragment), pCIT4233 (*AGL4*, *SspI–HpaI* fragment), pCIT2299 (*AGL5*), pCIT4210 (*AGL6*, 3′ *XhoI–EcoRI* fragment), pCIT2280 (*AGL3*), and pCIT857 (*GPA1*; see Ma et al. 1990). To avoid cross-hybridization, the probes for *AGL1*, *AGL2*, *AGL4–AGL6* correspond to carboxy-terminal portion of the proteins (less conserved) and 3′ nontranslated regions. Hybridizations were done as before (Yanofsky et al. 1990). 40 pg (about the amount of *AG* mRNA present in the total flower RNA) of in vitro synthesized RNA from *AGL* cDNAs (except *AGL3*) were also spotted on the same filters. The following cDNAs (see Fig. 1A) were used to synthesize RNA with the respective polymerase: pCIT4219 (*AGL1*, T7), pCIT4221 (*AGL2*, SP6), pCIT4214 (*AGL4*, T7), pCIT3216 (*AGL5*, SP6) and pCIT3209 (*AGL6*, T7). The plasmids were linearized so that only the inserts were used as templates for RNA synthesis.

In situ analysis was performed according to a previously described procedure (Barker et al. 1988; G. Drews, J. Bowman, and E.M. Meyerowitz, unpubl.). Inflorescences with young buds (stages 1–12; Bowman et al. 1989; Smyth et al. 1990) and flowers before pollination were fixed, embedded, and sectioned. The sections were hybridized with ^{35}S-labeled RNA probes complementary to *AGL* mRNAs. The plasmids pCIT4219 (SP6, *XhoI*) and pCIT4221 (T7, *NdeI*) (Fig. 1A) were used for *AGL1* and *AGL2* probes, respectively.

Acknowledgments

We thank S. Kempin for help with RFLP mapping, L. Medrano for excellent technical assistance, G. Drews and J. Bowman for advice on the in situ hybridization experiments, D. Spector and L. Fields for help with photography of in situ slides, A. Tagle for assistance in preparation of this manuscript, and J. Bowman, L. Brockman, C. Chang, G. Drews, B. Hamilton, T. Jack, L. Sieburth, V. Sundaresan, A. van der Bliek, and D.Weigel for valuable comments on this manuscript. This work was supported by grants from the National Science Foundation (DCB 8703439) and the Lucille P. Markey Charitable Trust. H.M. was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation, and M.F.Y. was supported by a postdoctoral fellowship in plant biology from the National Science Foundation (DCB 8608456).The sequence data described in this paper have been submitted to the GenBank database, and the accession numbers are *AGL1*, M55550; *AGL2*, M55551; *AGL4*, M55552; *AGL5*, M55553; and *AGL6*, M55554.

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